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STUDIES

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OF THE

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COLUMBIA UNIVERSITY
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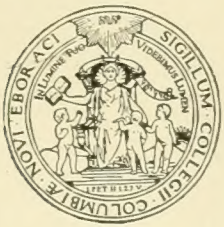

VOL. XIV

1914

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REPRINTS

PREFACE.

The papers included in this volume represent the results of the work done in the Department of Pathology of the College of Physicians and Surgeons during the collegiate year 1913-14.

The cost of reprinting and issuing the studies is defrayed by a special grant from Columbia University and not as heretofore by the Alumni Association of the College of Physicians and Surgeons.

W. G. MacCallum, M.D.,
Director.

Department of Pathology
College of Physicians and Surgeons
Columbia University, New York
June, 1914

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FURTHER EXPERIMENTAL STUDIES ON TETANY.

[Reprinted from the Journal of Experimental Medicine, Vol. XVIII, No. 6, 1913.]

FURTHER EXPERIMENTAL STUDIES IN TETANY.*

By W. G. MacCALLUM, M.D., and KARL M. VOGEL, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

In a paper read before the German Pathological Association at the meeting in Strassburg in 1912, I showed that the galvanic hyperexcitability of the nerves, which is a characteristic feature of tetany, is due to a change in the blood following the destruction of the parathyroid glands. It was demonstrated that after section of a nerve during tetany the isolated extremity remained hyperexcitable, and that such an isolated extremity became hyperexcitable if extirpation of the parathyroids was carried out after the cutting of the nerve. Then by connecting the blood vessels of an animal in the height of tetany with those of the leg of a normal animal so that the peripheral portion of the nerves of the limb of the normal animal were bathed in tetany blood, it was demonstrated that an excitability identical with that found during tetany appears very quickly in those nerves, and as quickly gives place to the normal conditions, when the femoral vessels are reunited with their stumps so that the nerves are again supplied with normal blood. In the same way the flooding of one leg of an animal in tetany with normal blood reduces the excitability of the nerves of that leg to the normal while the rest of the animal is in tonic and clonic convulsions.

The restoration of the femoral vessels to their original connections, so that the animal's own blood again flows through the leg, brings that extremity back to the condition of the others, and hyperexcitability of the nerves and twitching are again seen there. Similarly the blood removed from an animal during the convulsions of tetany and defibrinated may be perfused through the vessels of the amputated leg of a normal animal, whereupon its nerves become

* Received for publication, July 2, 1913.

hyperexcitable. If normal blood and tetany blood be used alternately in this perfusion the nerves will become correspondingly normally excitable or hyperexcitable. That this is not due to any mechanical disturbance of the circulation was shown by numerous experiments.

It is evident, therefore, that after the extirpation of the parathyroid glands there appears gradually a profound change in the blood which makes it capable of acting upon the nerves or their terminations in such a way as to make them respond to electrical stimuli far more rapidly than is normal. Doubtless this statement may be applied to the nerve cells of the spinal cord and brain as well as to those of the sympathetic system, but no ready means of demonstrating this has been devised.

It seemed, therefore, that since one might obtain in a bottle blood capable of producing this characteristic change in the excitability of the nerves, it should be possible to determine precisely the nature of the change and in that way throw light more directly upon the function of the parathyroid gland. For if it is the function of that gland to prevent the appearance of this change in the blood, it should be possible to penetrate further into the activities of this typical organ of internal secretion by discovering what chemical changes have occurred in the blood when the gland is destroyed.

Some years ago upon finding that tetany in animals and man could be interrupted at once by injections of a salt of calcium, Voegtlin and I suggested and upheld the idea that the glands in question might control the metabolism of calcium so that after their loss a serious disturbance in the calcium content of the blood might take place. This view was strengthened by the discovery that the blood of animals during tetany is distinctly poor in calcium and not especially weakened by the fact that other substances such as strontium and magnesium will also cut short the tetany. Undoubtedly when administered in this way the effect of such substances is to decrease the excitability of the nerves, and it is plain, therefore, that the effect would be the same no matter what substance might be responsible through its absence for the hyperexcitability.

The evidence at our command has not been sufficient to enable us to say with certainty what is the nature of the essential change in

the blood in tetany, and this is the problem which I have kept before me in the work of the past winter. It is known that the blood drawn from an animal in tetany differs from normal blood in its ability to cause hyperexcitability of the nerves with which it comes into contact, but it is still a matter of great difficulty to analyze this blood and to determine directly what change in its composition is responsible for its new properties. Any chemical procedure, even one so harmless, to all appearances, as the addition of Ringer's solution, will unfit the blood for perfusion since the nerves quickly die and lose their excitability when bathed with anything but fresh intact blood. Very indirect methods must therefore be used, and as yet it cannot be said that the matter is cleared up.

Three possibilities presented themselves for study, although of course there may be many more.

(1) The blood in tetany may contain a substance somewhat like strychnia in its mode of action, which may affect the nerves directly and increase their excitability.

(2) The blood may lack the normal amount of calcium and in that way cause the nerves to become hyperexcitable.

(3) The poison circulating in the blood may be of the nature of an oxalate and act by precipitating and thus withdrawing calcium from the tissue.

The simplest way in which this group of possibilities might be tested ought to be through the intravenous administration of an extract of the parathyroid gland. If this can counteract the effect of destruction of the parathyroids it must act upon the substances indicated in the first and third of the above possibilities, since the extract of the parathyroid could hardly contain enough calcium to be curative in the case of the second possibility. If then the addition of parathyroid extract to the tetany blood used in perfusion should eliminate its exciting effect, there must be a strychnia-like poison there, or else a substance capable of throwing out of action the calcium normally present. If not it would seem most probable that the blood had been simply deprived of its calcium.

Several experiments of this sort were made, of which the following will be recorded.

Dog 1,250. (Text-figure 1.) Excitability normal. Perfusion with tetany blood started at 12.50 P. M. Parathyroid extract (cow) added in small amounts (3 to 8 c.c.) at short intervals from 1.10 to 2.55 P. M., in all 40 c.c. to about 1,000 c.c. of blood.

	12.30	1.00	1.10	1.18	1.20	1.25	1.40	1.48	1.45	1.50	2.00	2.15	2.20	2.30	2.45	2.50	3.00	3.10	3.20	3.28	3.32	3.48
KC[1] . . .	0.5	0.5	0.1	0.05	0.1	0.1	0.05	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.5	0.1	0.1	0.1	0.2	0.2	0.1	0.4
KO . . .	Negative at 3.0	1.6	0.6	0.4	0.4	0.4	0.6	0.8	1.0	0.8	0.8	1.0	2.2	0.8	1.0	0.8	0.8	0.8	1.0	0.8	1.2	3.0
AC . . .	0.6	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.4	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.8
AO . . .	2.6	0.8	0.6	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.6	0.8	0.4	0.5	0.3	0.5	0.5	0.5	0.5	0.5	1.4
				a		*a*	*a*		*a*		*a*	Normal blood	*a*			*a*			Calcium chloride injected			

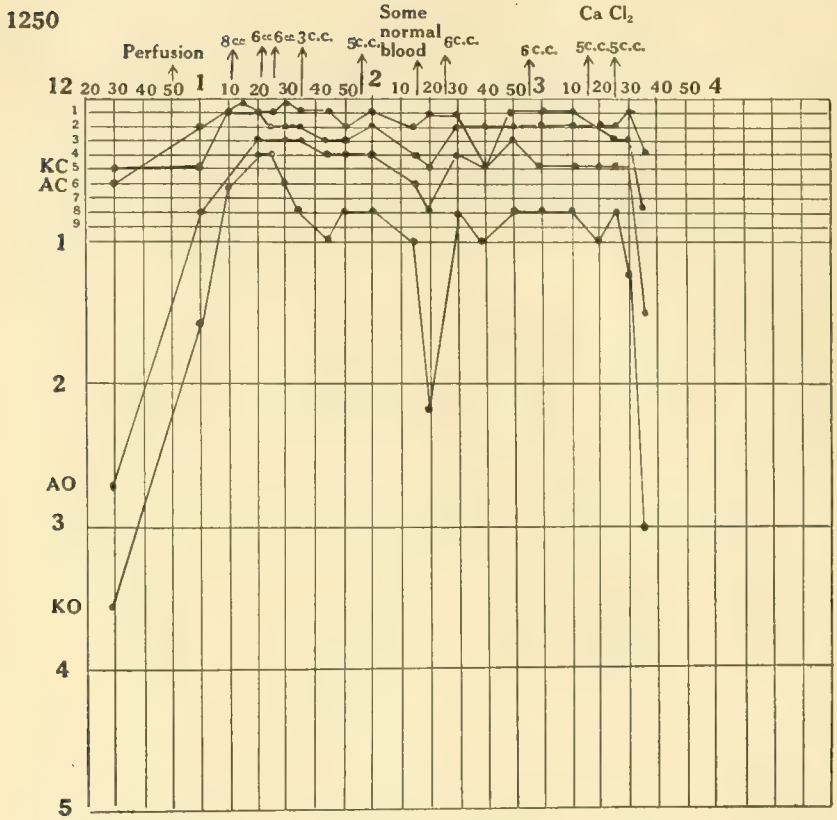
Parathyroid extract injected at *a*.

Dog 1,308. (Text-figure 2). Normal excitability. Perfusion with normal blood complete at 5.10 P. M. Tetany blood started at 5.30 P. M. 25 c.c of parathyroid extract injected at intervals in doses of 5 c.c.

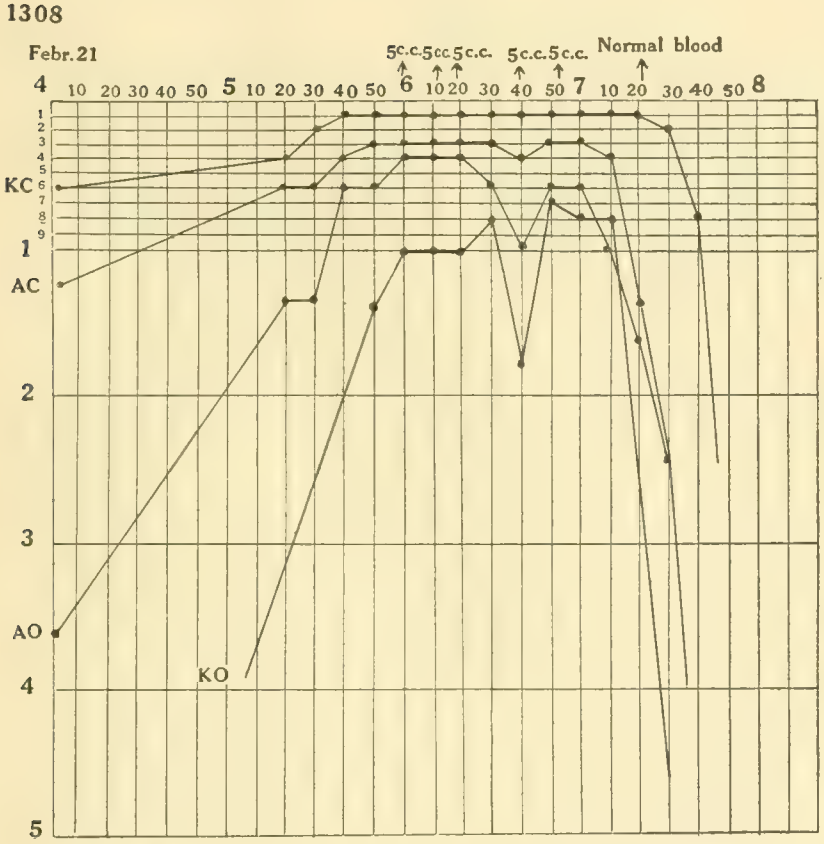
	4.00	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.40	6.50	7.00	7.10	7.20	7.30	7.45	7.48
KC	0.6	Perfusion complete.	0.4	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.8	Negative at 4
KO	Negative at 4.0		Negative at 5.0	Negative at 5.0	Tetanus at 2.0	1.4	1.0	1.0	1.0	0.8	1.8	0.7	0.8	0.8	Negative at 3.0	Negative at 5.0	Negative at 5.0	
AC . . .	1.2		0.6	0.6	0.1	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	1.4	2.4	4.0	
AO . . .	3.6		1.4	1.4	0.6	0.6	0.4	0.4	0.4	0.6	1.0	0.6	0.6	1.0	1.6	2.4	4.0	5.0
							a	*a*		*a*		*a*						

Parathyroid extract injected at *a*.

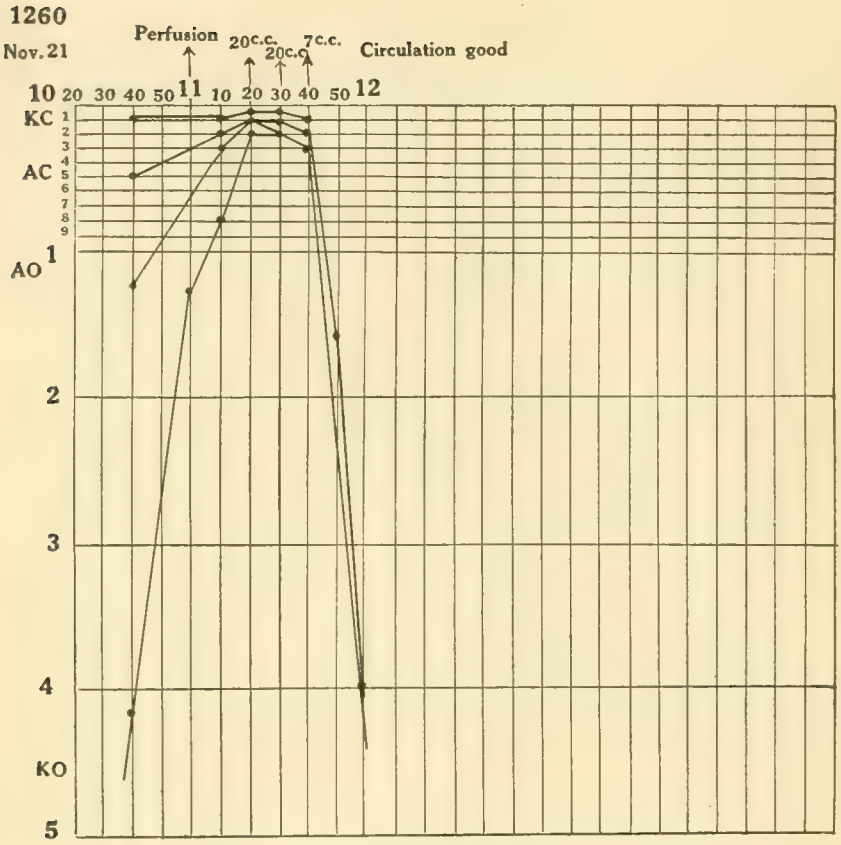
[1] KC = kathode closing; AO = anode opening, etc.



TEXT-FIG. 1. Perfusion of leg with tetany blood. Parathyroid extract added at intervals.



TEXT-FIG. 2. Normal leg perfused with tetany blood plus parathyroid extract, later with normal blood.



TEXT-FIG. 3. Perfusion of leg with tetany blood. Large doses of parathyroid added. Nerves injured.

The parathyroid extract seems to have little effect unless a large dose is given, when it seems capable of killing the nerves, as is shown in dog 1,260. The circulation was perfect throughout.

Dog 1,260.—(Text-figure 3.) Normal excitability. Perfusion at 11 A. M. with tetany blood to which there was added parathyroid extract as follows: 20 c.c. at 11.20 A. M., 20 c.c. at 11.30, and 7 c.c. at 11.40.

	10.00	11.00	11.10	11.20	11.30	11.40	11.50	12.00
KC.....	0.1	Perfusion complete.	0.1	0.05	0.05	0.1	1.6	4.0
KO.....	Negative at 4.0		1.2	0.2	0.2	0.3	1.6	4.0
AC.....	0.5		0.3	0.1	0.1	0.2	1.4	4.0
AO.....	1.2		0.3	0.1	0.2	0.5	1.4	4.0
				a	*a*	*a*		

Parathyroid extract injected at *a*. Circulation good.

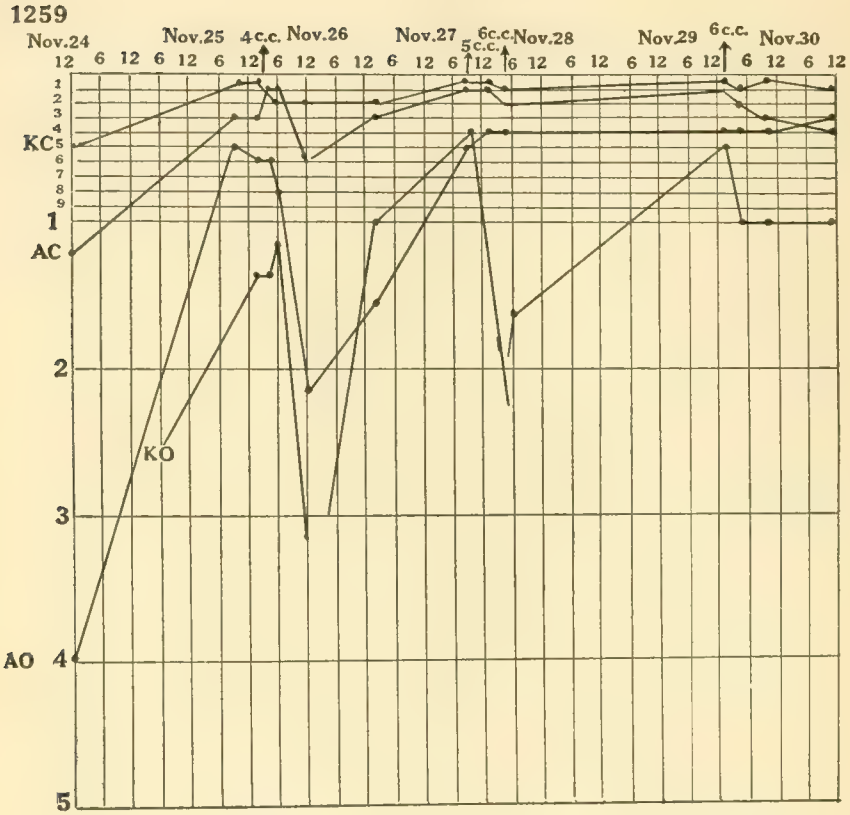
It was thought probable that since such tests last through a relatively short time their results might be untrustworthy, and, further, that in case the action of the parathyroid were directed against an oxalate-like substance there would be very little source for fresh calcium from the small amount of bony and other tissue in one extremity. For these reasons it seemed better to determine the effect of direct injections of parathyroid extract into the jugular vein of an animal in the height of tetany. Ten such experiments were made, all with practically the same result, so that only two or three will be recorded here. The extract of parathyroid glands used was made in some instances from the glands of the ox and extracted with Ringer's solution after being ground up fine with sand. The slightly blood-tinged filtrate from this suspension was used without any further treatment. In some of the experiments glycerin was added to the Ringer's solution which was used in the extraction. In other cases, since it seemed possible that the extract from ox parathyroid might be inefficient in the tetany of the dog, an extract was made from dog's parathyroids which, fortunately, were obtainable in large numbers. The same methods of extraction were used, and in one case 128 glands were extracted in fifty cubic centimeters, in the other seventy glands.

Such extracts, as well as the nucleoproteid precipitated from them by slight acidulation, have been used clinically to alleviate the symptoms of tetany and we have reported the apparent good effect in cases of experimental tetany when administered intravenously or subcutaneously. When they have been given by mouth I have not obtained any special effect, but that is the method employed in treating human beings, and beneficial results have been reported. The course of experimental tetany is notoriously irregular and remissions in its severity are common. Nevertheless, in earlier experiments I have been convinced, as I have in this later series, and as many others have reported, that distinct prolongation of the animal's life could be attained by the intravenous injection and that the twitchings and convulsions could be definitely diminished or even stopped. All these experiments were very rough, however, and no exact measure of the effect could be recorded. Now when we can measure exactly at least one feature of the disturbance, it is surprising to find that even enormous doses of parathyroid extract given intravenously do very little to lower the increased excitability of the nerves. It is true that in some cases there may be a temporary lowering but it is ephemeral, and in most instances all the readings remain up in the tetany level. Yet the animal shows a much decreased tendency to outspoken tetany and lives a good deal longer, so that the idea obtrudes itself that there may be another factor of importance which is different from that which produces the nervous hyperexcitability, or that the ganglion cells are more readily affected by the extract than the nerve terminations. The character of this effect will become clear in the following protocols and curves, but from them, and taking into consideration the enormous doses given directly into the vein, it seems questionable that the mere administration of small doses by mouth should really have the specific effects so often attributed to it.

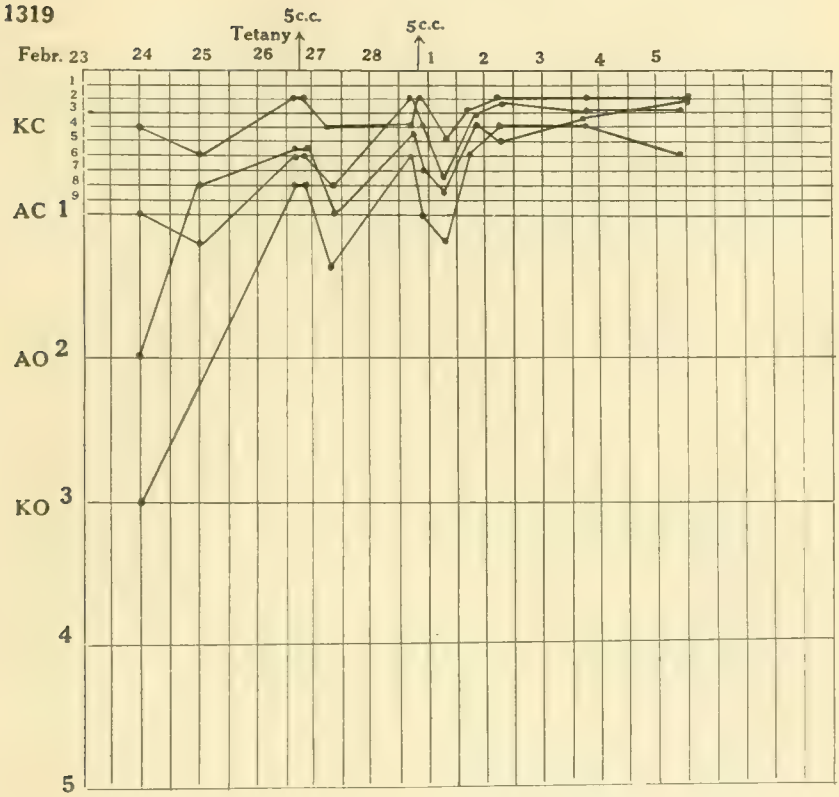
Dog 1,259.—(Text-figure 4.) Parathyroids removed November 24. Tetany not outspoken. Injections of extract of cow parathyroid (fifteen glands to 20 c.c. of Ringer's solution) as shown below.

	Nov. 21.	Nov. 24.	Nov. 25.					Nov. 26.			Nov. 27.			Nov. 29.				Nov. 30.
	12.00	12.00	11.00	2.30	3.20	5.00	5.45	12.00 M	3.30	10.30	12.45	5.30	5.45	2.45	3 30	4 45	9.00	1.00
KC.....	0.5	Operation.	0.05	0.05	0.05	0.1	0.2	0.2	0.2	0.05	0.05	0.1		0.05		0.1	0.05	0.1
KO.....	Negative at 3.0		Negative at 3.0	1.4	1.4	1.4	1.2	Negative at 3.0	1.0	0.4	Negative at 6.0	1.6		0.5		1.0	1.0	1.0
AC.....	1.2		0.3	0.3	0.1	0.2	0.2	0.6	0.3	0.1	0.1	0.2		0.1		0.2	0.3	0.2
AO.....	4.0		0.5	0.6	0.6	0.6	0.8	2.1	1.2	0.5	0.4	0.4		0.4		0.4	0.4	0.4
				a						*a*			*a*		*a*			

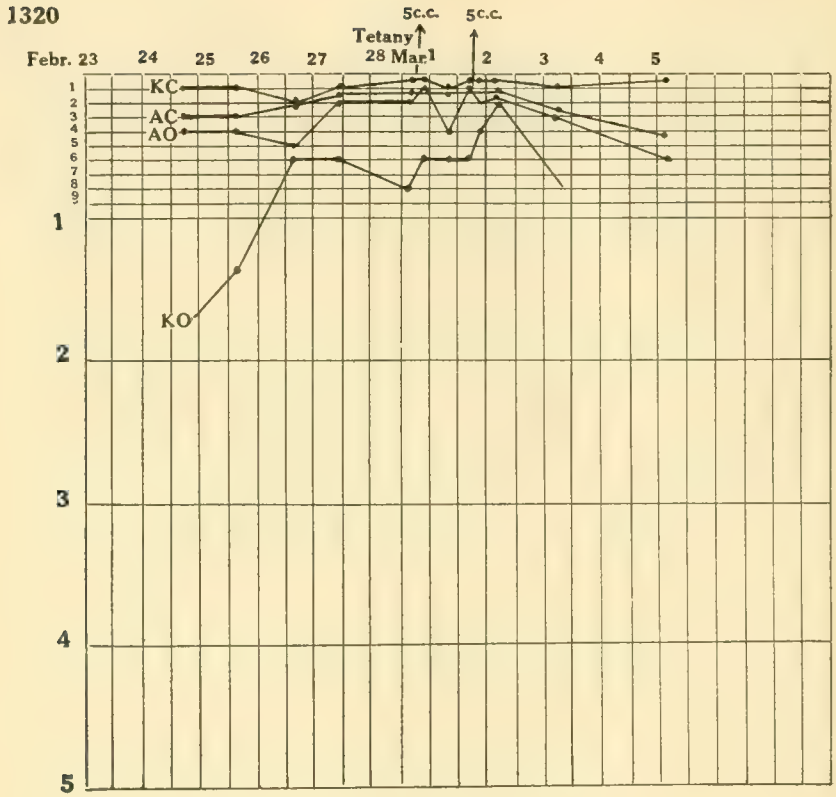
Parathyroid extract injected at *a*. Tetany on November 27 and 29.



TEXT-FIG. 4. Indefinite tetany. Injections of parathyroid extract.



TEXT-FIG. 5. Injections of parathyroid extract.



TEXT-FIG. 6. Tetany. Injections of parathyroid extract.

Dog 1,319.—(Text-figure 5.) Parathyroids removed February 23. Violent tetany February 26. Given two small doses of extract. Tetany disappeared or remained indefinite. Excitability decreased slightly after each injection but remained high.

	Feb. 24.	Feb. 25.	Feb. 26.		Feb. 27.	Feb. 28.		Mar. 1.		Mar. 2.	Mar. 3.	Mar. 4.	Mar. 5.
		2.40	3.45	4.30	11.30	4.00	5.00	10.00	2.30	11.30	5.15		12.20
KC.....	0.4	0.6	0.2	0.2	0.4	0.4	0.2	0.6	0.3	0.2	0.2		0.2
KO.....	3.0	Negative at 4.0	0.8	0.8	1.4	0.6	1.0	1.2	0.6	0.4	0.4		0.6
AC.....	1.0	1.2	0.6	0.6	0.8	0.2	0.4	0.8	0.3	0.2	0.3		0.3
AO.....	2.0	0.8	0.6	0.6 ă	1.0	0.4 ă	0.6	0.8	0.4	0.5	0.4		0.2

Parathyroid extract injected at *a*.

Dog 1,320.—(Text-figure 6.) Parathyroids removed February 23. Tetany distinct but not violent on February 28. Rather indefinite throughout the rest of the ten days' course, despite the high excitability. Two small doses of extract given without obvious result.

	Feb. 24.	Feb. 25.	Feb. 26.	Feb. 27.	Feb. 28.			Mar. 1.			Mar. 2.	Mar. 3.	Mar. 4.	Mar. 5.
					3.50	4.15	5.00	10.00	3.00	5.15				
KC...	0.1	0.1	0.2	0.1	0.05		0.05	0.1	0.05	0.05	0.05	0.1		0.05
KO...	—	1.4	0.6	0.6	0.8		0.6	0.6	0.6	0.4	0.2			Indefinite at 3.0
AC...	0.3	0.3	0.2	0.1	0.1		0.1	0.2	0.1	0.1	0.1	0.3		0.4
AO...	0.4	0.4	0.5	0.2	0.2	ă	0.1	0.4	0.1 ă	0.2	0.2	0.3		0.6

Parathyroid extract injected at *a*.

Dog 1.326.—Parathyroids removed March 17. Tetany distinct on March 19 and twitchings continued, sometimes amounting to definite tetany. Killed March 24. Parathyroid extract given at intervals in doses of 5 c.c. In all, 20 c.c. were given with very little effect. Tetany distinct March 20 and 21.

	Mar. 18.	Mar. 19.	Mar. 20.							Mar. 21.									Mar. 22.		Mar. 23.	Mar. 24.
	3.00	4.30	2.30	3.45	5.00	7.00	8.00	9.00	11.00	1.00	3.00	5.00	7.00	9.00	11.00	1.00	3.00	5.00	9.00	12.00	2.00		5.30
KC.	0.1	0.1	0.05		0.05	0.05		0.05	0.05	0.2	0.1	0.2	0.2		0.2	0.1	0.1	0.1	0.05	0.3	0.2		0.05
KO.	1.4	1.4	0.1		0.1	0.2		0.2	0.3	0.6	0.4	0.4	0.5		0.5	0.4	0.2	0.1	0.3	0.4	1.1		0.3
AC.	0.6	0.6	0.1		0.1	0.2		0.1	0.1	0.3	0.2	0.3	0.2		0.1	0.2	0.1	0.1	0.1	0.4	0.5		0.3
AO.	0.7	0.5	0.2		0.2	0.2		0.3	0.3	0.5	0.3	0.3	0.4		0.2	0.2	0.2	0.2	0.3	0.6	0.5		0.4
				a			*a*					*a*					*a*		*a*				

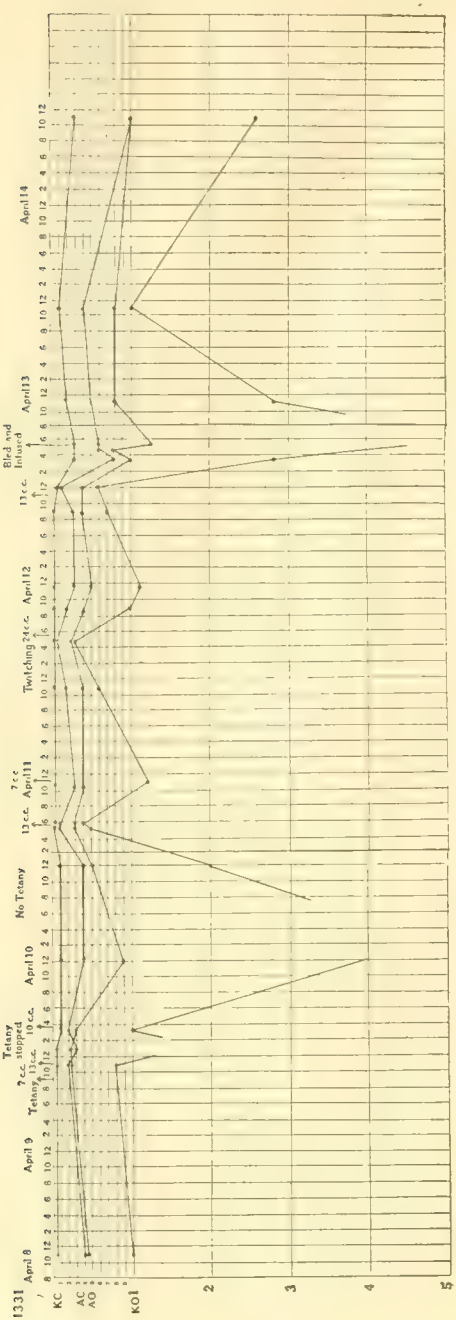
Parathyroid extract injected at *a*.

Larger doses were now used, as is shown in the following protocols.

Dog 1,334.—(Text-figure 7.) Parathyroids removed April 6. Violent tetany on April 9. Given doses of fresh extract from cow parathyroids ranging between 7 and 24 c.c. The extract was made with eighteen glands to 50 c.c. of Ringer's solution, and in such doses it seemed to have some effect on the excitability of the nerves. Even here, however, the effect is slight and of brief duration.

	Apr. 8.	Apr. 9.			Apr. 10.				Apr. 11.						Apr. 12.				Apr. 14.	Apr. 14.
	11.00	8.30	10.30	1.30	4.15	12.30	12 N.	5.20	5.50	10.30	11.30	4.30	8.15	11.40	9.45	12.00	3.25	4.45	5.10	11.30	11.00	
KC.	0.05	Tetany	0.05	0.05	0.1	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.5	0.3	0.3	0.2	0.1	0.3
KO.	1.0		0.8	Negative at 6.0	1.0	Negative at 4.0	2.0	0.5	0.4	1.2	0.6	0.3	1.0	1.1	0.7	0.6	2.8	Negative at 4.0	Negative at 4.0	2.9	1.0	2.6
AC.	0.4		0.2	0.3	0.2	0.4	0.4	0.1	0.1	0.3	0.2	0.05	0.2	0.3	0.3	0.1	0.8	0.6	0.6	0.5	0.4	1.0
AO.	0.4		0.2	0.2	0.3	0.9	0.5	0.3	0.3	0.4	0.4	0.3	0.4	0.5	0.4	0.4	1.0	0.8	1.2	0.8	0.8	1.0
					d			*d*			*d*		*d*		*d*			*B*				

Parathyroid extract injected at *d*. At *B* 400 c.c. of blood were removed and 400 c.c. of normal blood injected.

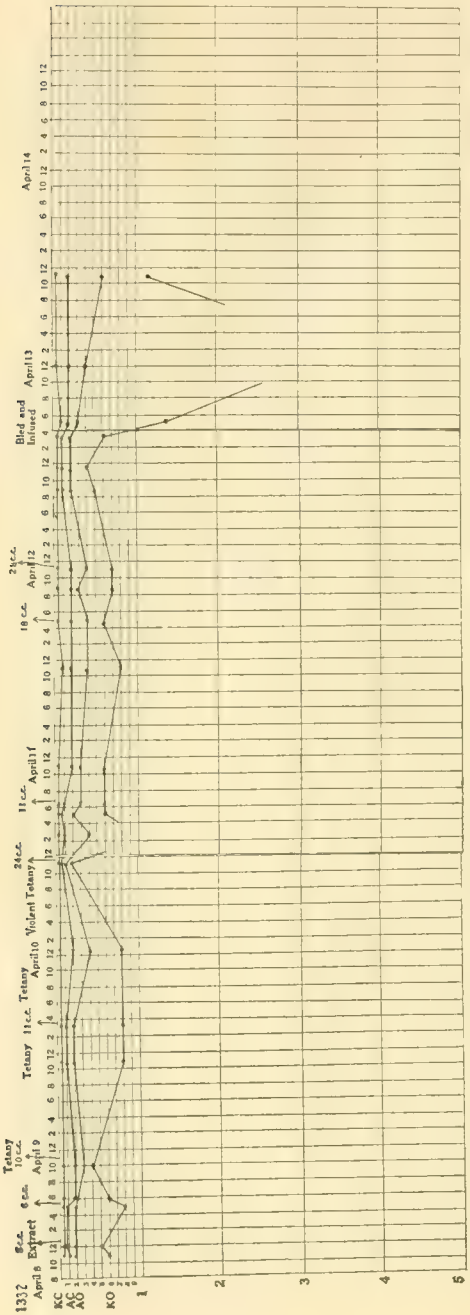


TEXT-FIG. 7. Tetany. Repeated injections of parathyroid extract. Finally bleeding with replacement by normal blood.

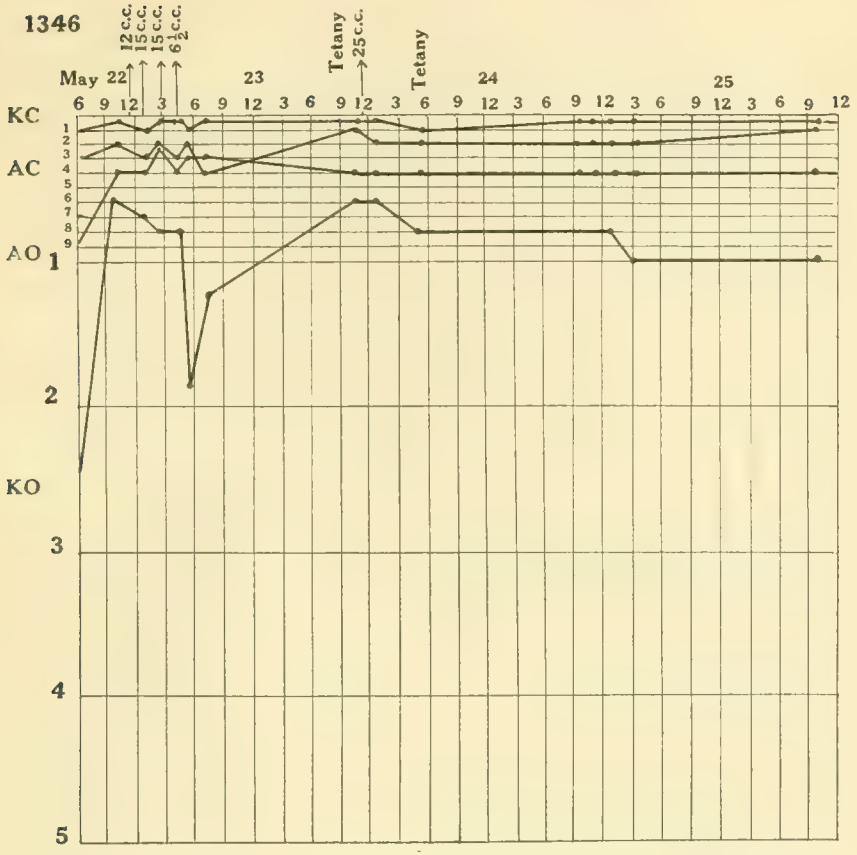
Dog 1,332. (Text-figure 8.) Parathyroids removed April 6. Slight tetany began April 8. Given extract of dog's parathyroid, later of cow parathyroid; about twenty glands from the dog were injected. Extract from ox parathyroid made from eighteen glands in 50 c.c. of Ringer's solution. 12 to 24 c.c. were given at each dose. The effect in all cases was very slight, although the animal was kept alive nearly a week. On April 12 bled 400 c.c. which was replaced with 400 c.c. of normal blood.

	Apr. 8.				Apr. 9.				Apr. 10.				Apr. 11.					Apr. 12.						Apr. 13.
	11.00	12.00	5.00	6.00	10.00	11.00	3.30	12.35	11.30	2.45	5.20	6.10	10.30	11.20	4.20	8.30	11.30	9.50	11.55	3.25	4.30	5.00	12.00	11.00
KC...	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05		0.1	0.05	0.05
KO...	0.6	0.5	0.8	0.6	0.4	0.8	0.8	0.8	0.2	Negative at 2.0	0.6	0.6	0.6	0.8	0.6	0.7	0.7	0.5	0.4	0.6		1.4	Negative at 4.0	1.2
AC...	0.1	0.05	0.05	0.2	0.2	0.1	0.1	0.2	0.05	0.1	0.05	0.01	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1		0.2	0.2	0.2
AO...	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.4	0.1	0.4	0.2	0.3	0.3	0.4	0.4	0.3	0.4	0.2	0.2	0.2		0.3	0.4	0.6
			a	*a*			*a*	*a*												*a*	*B*			

Parathyroid extract injected at *a*. Bled and transfused with normal blood at *B*.



TEXT-FIG. 8. Tetany. Repeated injections of parathyroid extract. Finally bleeding; replacement of blood with normal blood.



TEXT-FIG. 9. Tetany. Repeated injections of extract of dog's parathyroid.

It was feared that the slight effect of the injections of parathyroid extract might be due to the fact that ox parathyroids were used, and therefore an effort was made to obtain a sufficient number of the parathyroid glands of dogs to enable us to test the influence of an extract of those glands upon the tetany of a dog. In each instance about 100 glands were extracted in fifty cubic centimeters of Ringer's solution (without calcium.) In one case (dog 1,346) this fluid alone was used in extracting. In another (dog 1,350) glycerin was added. In neither instance did the effect seem greater than in the case of the cow parathyroid.

Dog 1,346.—(Text-figure 9.) Parathyroids removed May 19. Tetany on May 22. Large doses of extract were given at intervals, but the twitching persisted throughout the five days during which the dog lived.

	May 19.	May 20.	May 22.						May 23.			May 24.			May 25.		May 27.
			11.00	1.10	2.20	4.15	5.45	7.30	11.00	12.45	5.10	8.30	10.45	1.00	3.00	6.00	11.00
KC	0.2	0.1	0.05	0.1	0.05	0.05	0.1	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.08
KO	Negative at 5.0	Negative at 5.0	0.6	0.7	0.8	0.8	1.8	1.3	0.6	0.6	0.8	0.8	0.8	0.8	1.0	1.0	1.0
AC	0.5	0.5	0.2	0.3	0.2	0.3	0.2	0.4	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2
AO	2.0	1.0	0.4	0.4	0.2	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
			a	*a*	*a*	*a*			*a*								

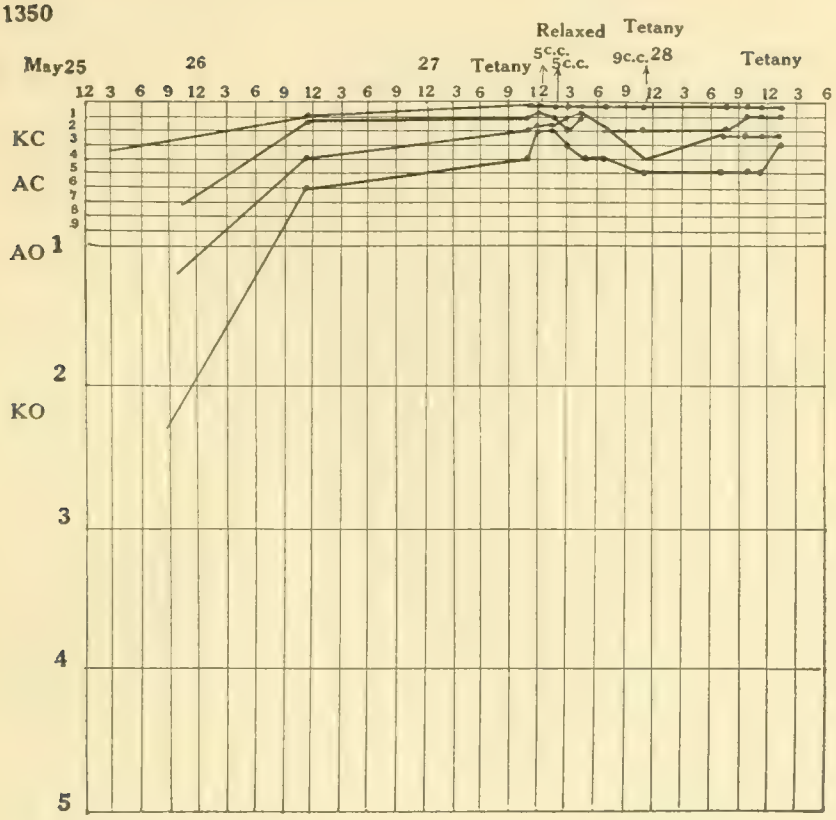
Parathyroid injections at *a*.

Dog 1,350.—(Text-figure 10.) Parathyroids removed May 25. Tetany varies and is sometimes outspoken; sometimes there is only a slight twitching in spite of the large doses of dog parathyroid.

	May 26.	May 27.							May 28.			
	11.30	11.00	12.00	2.00	3.00	4.30	6.15	11.00	8.20	9.30	11.00	1.00
KC..	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO..	0.6	0.4	0.2	0.2	0.3	0.4	0.4	0.5	0.6	0.5	0.5	0.3
AC..	0.1	0.1	0.05	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1
AO..	0.4	0.2	0.2	0.2	0.1	0.1	0.2	0.4	0.2	0.2	0.2	0.2
				a	*a*			*a*			(Violent tetany)	

Parathyroid injections at *a*.

The result of these experiments seems to show that even where relatively large doses of an extract of fresh parathyroid glands are administered in what would seem the most natural and most advantageous method (intravenously) to animals in tetany, the hyperexcitability of the nerves is not reduced at all or is lowered only temporarily and slightly, returning in a few hours to the original high level. Nor does it seem possible to keep an animal alive indefinitely by these injections when the parathyroid glands have been destroyed, although it is clear that life is distinctly prolonged



TEXT-FIG. 10. Tetany. Injection of parathyroid extract into vein.

thereby. One is disposed to ask whether there may not be some other feature of tetany apart from the hyperexcitability of the nerves which is beneficially affected by the extract.

The effect upon the hyperexcitability of the nerves is peculiar also inasmuch as the convulsions and twitchings practically disappear, and an animal found in complete rigidity with violent clonic contractions and tachypnea may be relieved and kept alive in a relaxed condition by the injection, although the electrical excitability of the peripheral nerves is practically unaltered. This is very different from the effect of injecting calcium under the same circumstances, for then the disappearance of the convulsions seems to be explained

by the extreme dampening of the excitability of the nerves. One can only imagine that in the case of the parathyroid injection the excitability may be lowered just below the threshold at which convulsions may occur. But the charts do not uphold this and there are many occasions when the electrical record is very high, though no twitchings are visible, while a few hours later with rather lower excitability there may be violent convulsions (text-figure 10).

Another possibility suggests itself, namely, that the excitability of the ganglion cells which send forth the impulses producing convulsions may be more readily affected by the parathyroid extract than that of the peripheral nerves. Such a condition would explain very well the rather curious effect of the extract. Even so the problem remains as to whether the death of the parathyroidectomized animal is the result of the mere mechanical exhaustion from the convulsions or of a general intoxication.

These experiments are unsatisfactory in throwing light on the question which we have set ourselves to decide,—whether the alteration in the blood in tetany is a mere loss of calcium, the presence of an oxalate-like substance capable of precipitating or rendering inactive the normal calcium, or the presence of a new poison somewhat like strychnia. The results might be compatible with the first view or even with the second if it proved that after neutralization of the oxalate-like substance the body failed to furnish enough fresh calcium to the tissues to make up the loss. Of course it may be that the extracts are faulty and inert in comparison with the activity of even the slightest amount of living parathyroid tissue. It may be that the gland acts not by way of any secretion but through its influence upon substances brought to its living cells by the circulating blood. And for that reason it may be that we are not really testing the third possibility with which these results seem so incompatible. A poison directly affecting the nerve cells might require living gland tissue for its destruction. Against this stands the fact brought out in the last paper that the blood of a normal animal will reduce the excitability of the nerves to normal after it has been heightened by tetany blood. Of course this in turn might be due to the presence of a sufficient amount of fresh calcium in the normal blood and the question is again unsettled. It might be

thought that the matter could be decided by bleeding an animal in tetany and restoring calcium-free Ringer's solution, and although this has been done and results as a matter of fact in the lowering of the excitability, it does so also in normal animals and therefore is not specific. Possibly it is due to a disturbance of the nutrition of the nerves. A few protocols will illustrate this point.

Dog 1,321.—(Text-figure 11.) Normal animal with average excitability. Bled 325 c.c. Blood replaced by 500 c.c. of calcium-free Ringer's solution. Excitability tested before and each hour after this operation.

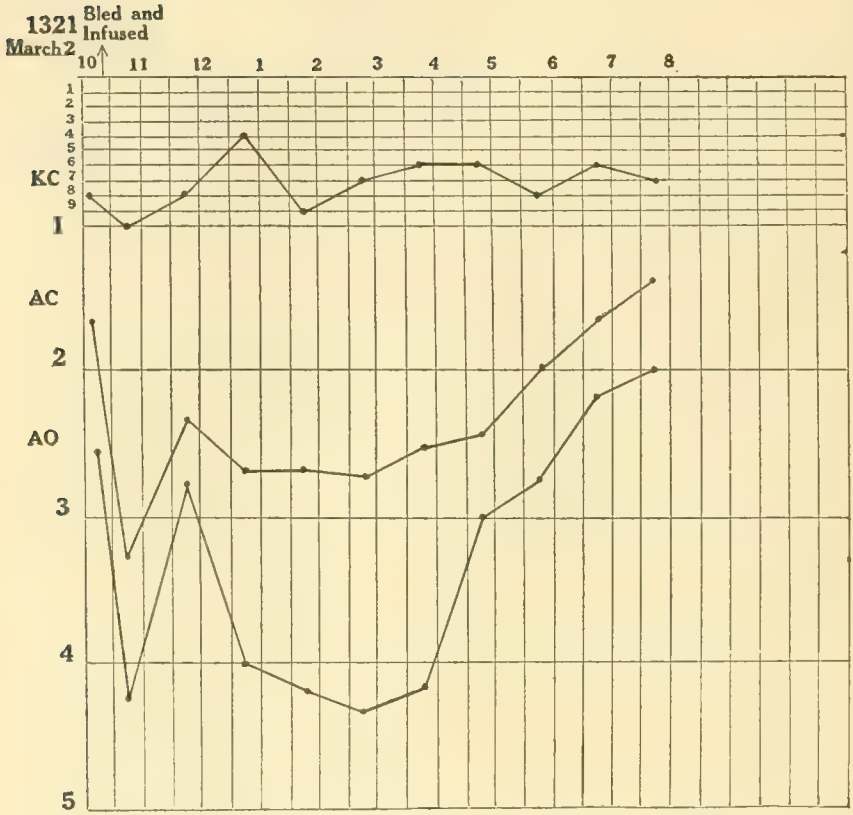
	Mar. 2.											Mar. 3.
	10.10	10.15	10.45	11.45	12.45	1.45	2.45	3.45	4.45	5.45	6.45	7.45	
KC ...	0.8	Bled and infused.	1.0	0.8	0.4	0.9	0.7	0.6	0.6	0.8	0.6	0.7	0.4
KO ...	Negative at 5.0		—	—	—	—	—	—	—	—	—	—	—
AC	1.6		3.2	2.4	2.6	2.6	2.7	2.5	2.4	2.0	1.7	1.4	1.2
AO	2.6		4.2	2.8	4.0	4.2	4.4	4.2	3.0	2.8	2.2	2.0	3.4

Dog 1,324.—Large normal dog; weight 30 kilos. Bled 630 c.c. from carotid. 1,400 c.c. of Ringer's solution without calcium were run into the jugular vein.

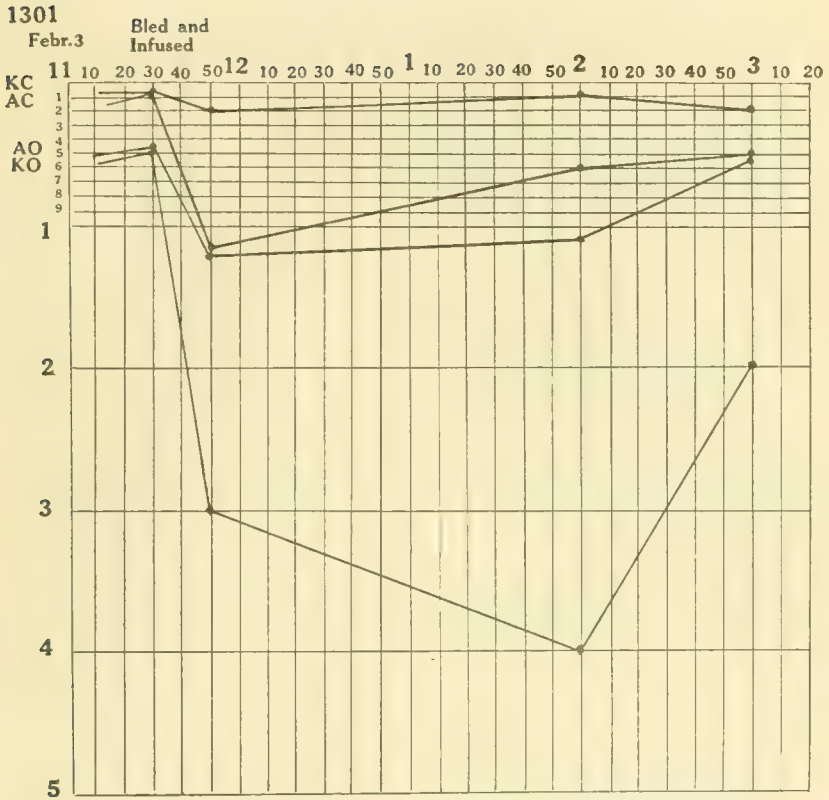
	Mar. 3.				
	12.00	12.15	12.30	2.00	5.30
KC	0.3	Bled and infused.	0.3	0.3	0.3
KO	Negative at 5		—	—	—
AC	0.6		1.0	1.6	1.9
AO	1.2		2.4	2.8	3.0

Dog 1,301.—(Text-figure 12.) Parathyroids removed January 31. Violent tetany February 3, 11.30 A. M. Bled 450 c.c. 250 c.c. of Ringer's solution without calcium were injected. Died at 5 P. M.

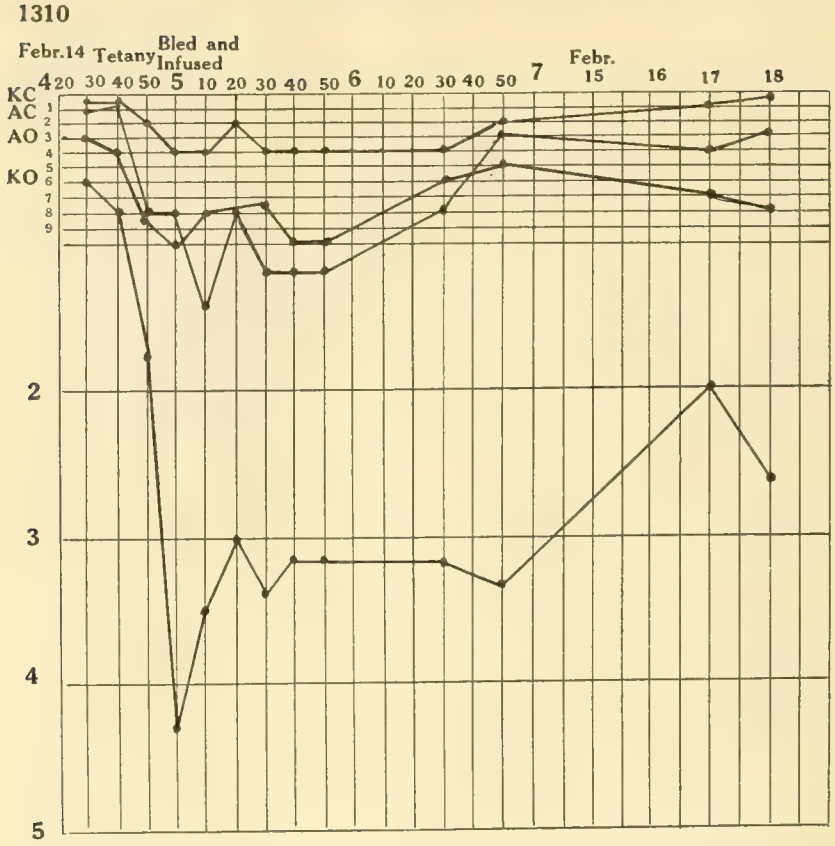
	Feb. 3.				
	11.30	11.40	11.45	2.00	3.00
KC	0.05	Bled and infused.	0.2	0.1	0.2
KO	0.5		3.0	4.0	2.0
AC	0.05		1.2	0.6	0.5
AO	0.5		1.2	1.1	0.5



TEXT-FIG. 11. Bleeding followed by injection of Ringer's solution without calcium.



TEXT-FIG. 12. Tetany. Bled 450 c.c.; 250 c.c. Ringer's solution without calcium returned.



TEXT-FIG. 13. Tetany. Bled 500 c.c.; 1,200 c.c. Ringer's solution without calcium in vein.

Dog 1,310.—(Text-figure 13.) Parathyroids removed February 9. Tetany distinct February 14, 4 P. M. Bled 500 c.c. at 4.50 P. M. 1,200 c.c. of Ringer's solution without calcium were injected into the jugular at 5.10 P. M.

	Feb. 14.												Feb. 15.	Feb. 16.	Feb. 17.	Feb. 18.
	4.30	4.40	4.50	5.00	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.50				
KC	0.05	0.05	0.2	0.4	0.4	0.2	0.4	0.4	0.4				0.4	0.2			0.1	0.05
KO	0.6	0.8	1.8	4.4	3.0	3.0	3.4	3.2	3.2				3.2	3.4			2.0	2.6
AC	0.1	0.5	0.8	0.8	1.4	0.8	1.2	1.2	1.2				0.8	0.3			0.4	0.3
AO	0.3	0.4	0.8	1.0	0.8		0.8	1.0	1.0				0.6	0.5			0.7	0.8
		Bled			Infused													

Dog 1,314.—(Text-figure 14.) Parathyroids removed February 18. High excitability but not violent tetany on February 21. At 3.40 bled 500 c.c. 1,000 c.c. of Ringer's solution without calcium were introduced.

	Feb. 20.	Feb. 21.					Feb. 22.
		3.30	3.50	4.45	5.45	7.45	11.15
KC.....	0.1	0.05	0.4	0.4	0.4	0.2	0.05
KO.....	Negative at 3.0	0.6	3.0	2.0	Negative at 3.0	4.0	2.0
AC.....	0.2	0.1	1.2	0.6	0.8	0.8	0.4
AO.....	0.4	0.3	1.0	1.0	1.4	1.4	0.6

One feels almost sure from these experiments that the method is too coarse to throw any light upon the nature of the alteration of the blood in tetany. The excitability of the nerves, as we have seen from perfusion experiments and those concerned with anemia, is a property so delicate that it is readily disturbed or destroyed by measures which change in any marked degree the character or amount of the nutritive blood supply. It is probably for this reason that the symptoms of tetany may be relieved, as Meltzer[2] pointed out, by the injection of a great amount of strong salt solution, and not because of any specific action of the sodium chloride. That such effects pass off after a time is due doubtless to the power of the body to reëstablish the normal composition of the blood.

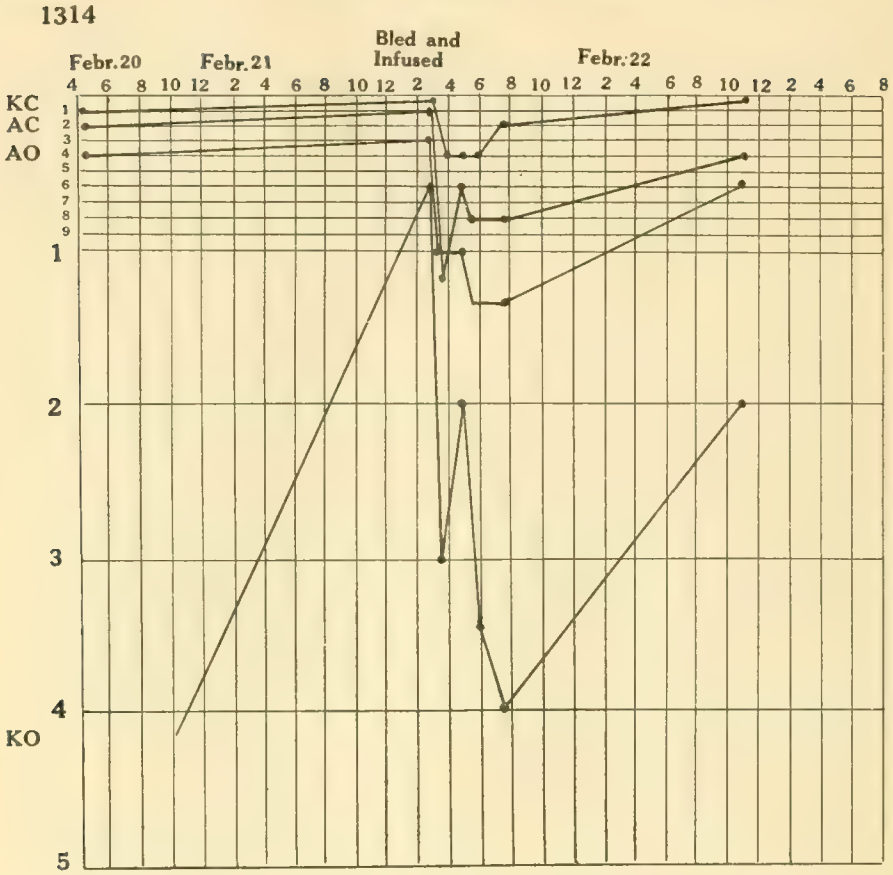
Other methods of attack which we have previously used have been employed again, and the results will be recorded here.

Since we know definitely that the blood of an animal in tetany differs from that of a normal animal, it would at first sight seem possible to study the blood directly, but the difficulties are very great. Direct analyses to show the calcium content can of course be made, and some have been recorded in a previous paper,[3] showing a marked decrease in the calcium content during tetany.

It was thought that chemical analyses might contribute something to the understanding of the action of the parathyroid extract, if we should determine any differences between the calcium contents of the blood of untreated dogs suffering from tetany and that of dogs

[2] Joseph, D. R., and Meltzer, S. J., *Jour. Pharmacol. and Exper. Therap.*, 1910–11, ii, 361. Voegtlin, C., and MacCallum, W. G., *ibid.*, p. 439.

[3] MacCallum, W. G., and Voegtlin, C., *Jour. Exper. Med.*, 1909, xi, 142.



TEXT-FIG. 14. Tetany? At 3 P. M. on February 21 bled 500 c.c., 1,000 c.c. of Ringer's solution returned.

which had received repeated doses of parathyroid extract. In other cases we analyzed the blood of dogs from which most of the parathyroid tissue had been removed because, as we supposed, some of the gland tissue had remained uninjured. There are therefore four categories into which these animals fall.

Normal.	Tetany.	Tetany treated with parathyroid extract.	Partial parathyroidectomy without tetany.
Dog 1,324—0.0624	Dog 1,349—0.0156	Dog 1,350—0.0200	Dog 1,344—0.0541
Dog 1,360—0.0731	Dog 1,352—0.0322	Dog 1,331—0.0264	Dog 1,345—0.0444
Dog 1,361—0.0580	Dog 1,348—0.0226	Dog 1,332—0.0436	
Dog 1,362—0.0502	Dog 1,353—0.0390		

These figures represent the content in calcium in grams of 1,000 grams of blood. The analyses were made by the method of Neumann, the calcium being estimated gravimetrically and not by titration with potassium permanganate. They were made without knowledge of the history of each case and compared only after all were finished. It is plain from these figures that the calcium content of the blood of normal dogs and of those which did not develop any symptoms of tetany even though the parathyroid gland tissue was much reduced is much higher than in the other two groups. It may be observed further that the great decrease in the amount of calcium in the blood of the animals which show tetany persists in spite of the injection of parathyroid extract, a point which is significant and in accord with the results of the electrical tests of nervous excitability. Probably we may conclude from this that the idea that parathyroid extract might stop the tetany by heightening in some way the calcium content of the blood is without actual foundation.

Several attempts were made in the direction of imitating the action of a possible oxalate-like substance in the blood by injecting a solution of sodium oxalate into the vein. In a previous paper it was shown that the addition of a minute quantity of sodium oxalate to the normal blood used in perfusing a limb caused a hyperexcitability of the nerves, but that it was difficult to carry out this experiment without killing the nerves. Sarvonat and Roubier[4] have shown that the prolonged administration of oxalates by mouth will finally produce such hyperexcitability which could be measured electrically. The injection of quite large quantities of the oxalate into the veins of an animal should produce such a change, but several experiments of this sort have given practically no result although the animal sometimes died during the injection. The solution was then allowed to run into the vein much more slowly, and although it caused vomiting and depression it produced no especial change in the excitability of the nerves. When, however, the same animal was again injected the next day or the day after, the effect became noticeable (text-figure 15).

[4] Sarvonat, F., and Roubier, Ch., *Arch. de méd. expér.*, 1911, xxiii, 584; *Jour. de physiol. et de path. gén.*, 1911, xiii, 565.

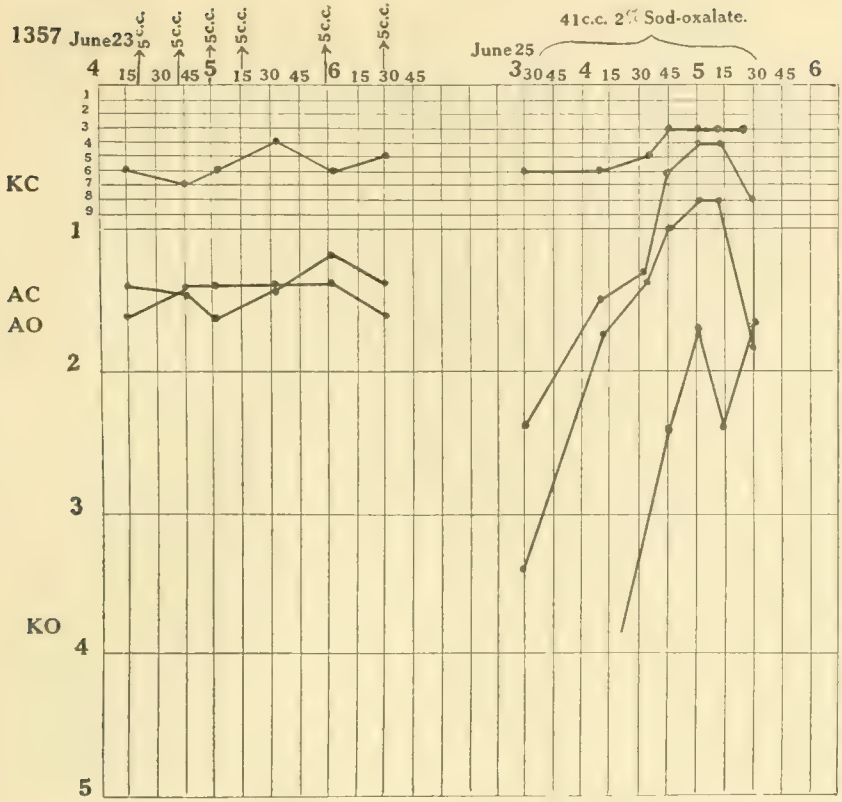
Dog 1,357.—(Text-figure 15.) Normal animal. On June 23 a 2 per cent. solution of sodium oxalate was slowly run into the vein so that several minutes were occupied in the introduction of 5 c.c. No especial effect was produced. 30 c.c. of the solution were administered. Two days later 41 c.c. of the same solution were run into the vein continuously but very slowly. This produced a distinct rise in the excitability of the nerves.

	June 23.						June 25.						
	4.15	4.45	5.05	5.35	6.05	6.30	3.30	4.10	4.35	4.45	5.00	5.10	5.30
KC.....	0.6	0.7	0.6	0.4	0.6	0.5	0.6	0.6	0.5	0.3	0.3	0.3	0.3
KO.....	Negative at 5.0	—	—	—	—	—	Negative at 5.0	Negative at 5.0	Negative at 5.0	2.4	1.8	2.4	1.8
AC.....	1.6	1.4	1.4	1.4	1.4	1.6	3.4	1.8	1.4	1.0	0.8	0.8	1.8
AO.....	1.4	1.4	1.6	1.4	1.2	1.4	2.4	1.6	1.4	0.6	0.4	0.4	0.8
		30 c.c. 2 per cent. oxalate.						41 c.c. 2 per cent. oxalate.					

It is important that more experiments lasting over a longer period should be carried out, since the body seems to be able to repair for a time the injury to the blood caused by the oxalate. It was quite remarkable to observe that the heart blood taken from a small dog which had died during the administration of the 2 per cent. oxalate after twenty-eight centimeters had run in, clotted firmly within five minutes. It seemed that this amount should be sufficient to prevent the clotting in a small dog weighing only about five kilograms, but apparently the circulating blood can protect itself. When the introduction of oxalate is repeated this protection seems to become less and less available, until finally the effect is observed in the nerves.

SUMMARY.

It has been our aim to determine in what particular the blood is altered during tetany so that it can produce hyperexcitability of the motor nerves. As a working hypothesis it has been assumed that (1) there may be a lack of calcium in the blood and tissues; (2) there may be a circulating poison which like an oxalate could render inactive the circulating calcium; and (3) there may be a substance in circulation vaguely resembling strychnia in its action upon the nervous system and directly causing the hyperexcitability. It is



TEXT-FIG. 15. Injection of 2 per cent. sodium oxalate into jugular vein.

impossible to decide with our present knowledge which, if any, of these explanations is the true one, but certain experimental results are brought forward. If tetany blood be used to perfuse a normal leg the excitability of the nerves rises to a characteristically high level and the addition of parathyroid extract to the blood has little or no effect in lowering this excitability.

Parathyroid extract whether from the ox or the dog fails when injected into the circulation of an animal in tetany to reduce the excitability of the nerves markedly or permanently, although it seems to affect the more sensitive ganglion cells thus cutting off excessive impulses to the periphery. Although the nerves remain hyperexcitable, tetany is usually much diminished or abolished

entirely. This seems to be analogous to the action of ether or any other anesthetic which may inhibit the activities of the ganglion cells, although it leaves the nerves excitable and able to conduct impulses. An animal in tetany relaxes instantly on being given ether although the excitability of the nerves to electric currents is little changed.

Bleeding followed by the replacement of the blood with an indifferent solution free from calcium stops tetany and lowers the excitability of the nerves. Probably this cannot be ascribed to the removal of a circulating poison, but rather to a general disturbance of the nutrition of the nervous system.

Oxalate-like substances introduced into the circulation rapidly, and for a short time only, may kill the animal, but they seem to produce no change in the excitability of the nerves. If the solution is injected very slowly and over a long period the protective action of the body seems to be overcome and the excitability of the nerves rises to high levels. This seems to resemble somewhat the latent period after the destruction of the parathyroid before tetany begins.

Direct analysis of the blood shows that as compared with the normal, the blood of an animal in tetany is very poor in calcium. Administration of parathyroid extract does not increase this calcium content. On the contrary, if the extirpation of the parathyroids has been incomplete so that tetany does not appear, the calcium content of the blood is that of the normal animal.

Even yet, therefore, in spite of our efforts to shake it, the theory that tetany is closely dependent upon a disturbance of the calcium content of the blood is supported by stronger evidence than any other idea, but much remains to be done before a clear conception of the process is reached.

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ON THE MECHANISM OF THE CIRCULATORY FAILURE IN DIPHTHERIA

By W. G. MacCALLUM, M.D.
NEW YORK

(From the Department of Pathology, College of Physicians and Surgeons New York

In the course of acute infections it is well known that the patient may die with symptoms somewhat like those of surgical shock. The blood-pressure falls, the pulse becomes weak and small, the skin is pale or cyanotic about the nose and ears, sweating and vomiting ensue, and with shallow respiration and general collapse the heart stops beating.

The discussion as to the mechanism of this disturbance is so well known that only the briefest résumé need be given here. It had long been thought from clinical observations that the affection might be one of the vasomotor apparatus rather than the heart itself and Romberg, Paessler, Brühns and Müller attempted to prove this in the case of diphtheria, pneumococcus, and pyocyaneus infections by experimental studies. In these studies they relied upon certain methods which aimed to demonstrate that the heart would regain its activity if the failure of the vasomotor apparatus were overcome in some way and that those things which ordinarily caused vasomotor reactions were ineffectual. Thus they found that the mechanical expression of the blood from the splanchnic veins into the heart would restore for a time the aortic pressure and with that the coronary blood supply and thus the nutrition and activity of the heart. The same could be produced by compressing the aorta. Barium chloride still caused the vessels to contract but sensory stimuli had lost their effect. They therefore concluded

that the circulatory failure was not due primarily to injury of the heart but rather to the paralysis of vasomotor centres in the brain which, causing a lowering of arterial resistance, quickly affected the heart throught the diminished coronary supply to the heart wall. Romberg especially pointed out the fact that if the heart were at fault all these tests should fail.

Then Stejskal criticised their methods, showing that abdominal massage could increase arterial pressure even in a dead animal and that its effects are very transient. He believed that the heart itself is directly injured and fails on that account. Gottlieb supported this view to some extent, believing that the withdrawal of blood from the coronary circulation need not kill the heart so rapidly since a heart may be kept for hours after the animal's death and then revived by perfusion. He therefore thought death probably due to respiratory stoppage with subsequent failure of the heart due to its injury by the poison.

Rolly and Paessler later reviewed the subject and seem to admit the force of Stejskal's objections as to the abdominal massage, and though they maintain the idea that the injury to the heart is through malnutrition, they state that it becomes very susceptible to other injuries. Other writers whose papers are referred to below lean to one or the other view or accept the idea that both vasomotor paralysis with its consequences and direct injury to the heart wall occur.

In the course of a study of the mechanical conditions present in aortic insufficiency, a plan was devised by which the influence of the vasomotors might be almost entirely eliminated and the work of the heart studied by itself.[1] This plan consisted in simply dividing the aorta near its root and connecting it with a long rubber tube ending in a curved glass outlet which could be elevated to any height upon a graduated upright pillar. The aortic pressure could in this way be set at any point according to the height to which the heart must drive the blood against the force of gravity. The blood which escaped fell into a cistern and returned through another long tube to the distal part of the aorta. Of course by applying this

[1] W. G. MacCallum, Johns Hopkins Hospital Bulletin, 1911, xxii, 197.

arrangement to an animal in the collapsed condition which follows poisoning with the diphtheria toxin the injurious effects of paralysis of the vasomotors would be removed at once, and if the theory of the Leipzig school be correct, the heart should return to its normal activity and the animal continue to live.

The following protocols will illustrate the results of this experiment:

Dog 1. Weight 8.9 kg., April 25, 1911, 1 A.M. Given 1 c.c. diphtheria toxin intravenously. At 11 A.M. the animal was found lying sick in the cage. Carotid pressure taken at 11.30. Chest opened and circulation arranged as described, the outlet being set at 130 cm. which was the carotid pressure before the operation. The heart beat well for two hours, after which there appeared great edema of the lungs and the heart dilated and stopped. All of the organs were found to be edematous and to show numerous ecchymoses. The heart beat regularly throughout and the output was well maintained.

Dog 6. Weight 6.6 kg., May 3, 1911., 12.45 A.M. Given 0.75 c.c. diphtheria toxin intravenously. At 1 P.M. carotid pressure taken, aorta cut and external circulation arranged. This finished at 1.20 and the heart beat well until 3.10, when there was extreme edema of the lungs and of other organs. In the heart, which was otherwise grossly normal, there were many hemorrhages. Here as in the first case the heart continued active for two hours.

Dog 7. Weight 10.3 kg., May 5, 1911, 12.30 A.M. Given 2 c.c. diphtheria toxin intravenously. At 12 noon dog was apathetic and drowsy. At 2 P.M. vomited and appeared very weak. Artificial or external circulation arranged at 2.40 and heart beat at a pressure of 110 cm. blood until 5.45. At times the heart began to collapse and beat with great excursions, driving out relatively little blood. One dose of digitalin and strychnine was given which restored it to its normal beat. During the latter part of the three hours there were violent general convulsions.

Dog 13. Weight 6 kg., April 2, 1913, 10.30 A.M. Given 3 c.c. diphtheria toxin intravenously. Found very sick at 4 P.M., and at 4.45 the external circulation was arranged after which the heart beat, quite well until 7.20, when the supply of blood failed and the experiment was stopped. There was some irregularity in the action of the heart, but on the whole it beat strongly for two hours and thirty-five minutes.

In order to have a good control observation two dogs of the same size were given the same dose at the same time. It was

intended to show that with the aid of the maintenance of arterial pressure one could be kept alive long after the other had died.

Dog 14 A. Weight, 10560; female.

Dog 14 B. Weight, 10900; male. Each given 4 c.c. diphtheria toxin intravenously at 8 A.M. April 3, 1913, both dogs had vomited and were depressed and weak. At 5 P.M. dog A was found lying stretched out stiffly and not breathing. Heart beating feebly. Artificial respiration was begun. Carotid pressure very low. It was attempted to establish the external circulation, but through an accident the dog died. By this time, 5.35, dog B was almost dead and lay inert. Reflexes were gone, the eyes rolled about, and there were convulsive twitchings. External circulation was established at 5.45 and tracings of the heart beat were taken until 8.35, a period of two hours and thirty five minutes, when the heart stopped apparently only because it finally became necessary to use blood which had been kept several days.

Dogs 15 A and B, weighing respectively 11 and 10.3 kg., were given 4 c.c. diphtheria toxin at 8 A.M., April 8, 1913. At 3.15 both dogs were almost dead, lying quite apathetic, with complete loss of reflexes and occasional convulsions. Dog A, whose blood-pressure as taken from the carotid was very low, about 60 cm. of blood, was arranged with the external circulation and the outflow tube set at 85 cm. The heart beat improved at once and continued from 3.30 to 4.40. In the meanwhile dog B died at 3.20. The character of the pulse of Dog A as seen in the tracing varied spontaneously, being rapid and strong at times and producing an abundant outflow, while at other times there was a slower collapsing beat which propelled relatively little blood to the outflow. After one hour and ten minutes it became distended during a convulsion, and could not be made to beat again. It seemed that in this case at least the heart itself had been injured by the poison.

For comparison normal dogs were subjected to the same operation as shown in the following protocols:

Dog 2. Weight, 6.9 kg. Anesthetized with chloretone April 28, 1911. External circulation complete at 3.30 P.M.; pressure set at 130 cm. blood, which equals about 100 mm. mercury. At 4.45 there was much oozing from cut tissues and heart became distended. Pressure lowered to 53 cm. and heart beat again. Some edema of lungs and of other tissues. Given a dose of digitalin, after which heart beat well until 6.30, when the dog was killed.

Dog 5. Weight, 9.9 kg. Anesthetized with ether May 2, 1911. The external circulation was arranged through one intercostal space at 3.20, and the outflow tube set at 130 cm. of blood. The heart beat well until 6.20, when the dog was killed.

The actual output per minute of each of these hearts cannot be accurately determined from the curves produced by this method because much of the blood circulates through the head and forelimbs, but since the records were all made in the same way it will give some idea of the character of the heart's work after diphtheria poisoning to compare the output from the artificial aorta with that in the case of the normal dogs.

Dog 7 (diphtheria). Weight, 10.3 kg. Outlet set at 110 cm.
Operation at 2.40.
At 3.30, 100 c.c. is delivered in twenty-six seconds.
At 4.00, 100 c.c. is delivered in twenty-eight seconds.
At 4.30, 100 c.c. is delivered in thirty-two seconds.
At 4.35, 100 c.c. is delivered in thirty seconds.
Dog 1 (diphtheria). Weight, 8.9 kg. Outlet at 130 cm.
Operation at 11.30.
At 12.00, 100 c.c. is delivered in eighteen seconds.
At 12.30, 100 c.c. is delivered in nineteen seconds.
At 1.00, 100 c.c. is delivered in nineteen seconds.
At 1.30, 100 c.c. is delivered in eighteen seconds.
Dog 2 (normal). Weight, 6.9 kg. Outlet at 130 cm.
Operation at 3.30.
At 3.50, 100 c.c. is delivered in twenty-one seconds.
At 4.30, 100 c.c. is delivered in twenty and a half seconds.
At 5.45, 100 c.c. is delivered in thirty-four seconds.
Dog 5 (normal). Weight, 9.9. Outlet at 130 cm.
Operation at 3.
At 3.45, 100 c.c. is delivered in thirty-five seconds.
At 4.30, 100 c.c. is delivered in twenty-nine seconds.
At 5.00, 100 c.c. is delivered in thirty seconds.
At 5.30, 100 c.c. is delivered in twenty-nine seconds.

From these it will be seen that the actual work of the diphtheria hearts is at least as good as that of the normal hearts.

It is probable that if the conditions were absolutely perfect and the exposure, use of defibrinated blood, etc., avoidable, it would be possible to keep a normal heart driving the external circulation for a longer time than three hours. It is probable, too, that even under the present circumstances a normal heart treated in the way the hearts of the poisoned animals were treated would beat a little longer and a little more steadily than they. But the difference is

so slight that, while it seems probable from such spontaneous irregularities as occurred in the case of dog 15 A that the heart itself is injured by a large dose of toxin, it is clear enough that it is still able to carry on the circulation for two or three hours against a high pressure—a thing which is vastly different from mere beating in a perfusion apparatus.

It might be objected that the use of fresh blood would restore the heart by washing away the toxin, but in all these experiments the blood of the animal itself was returned to the circulation and is merely diluted. At any rate, Decroly and Ronnse showed that diphtheria toxin injected into the blood stream disappears completely after four to seven minutes, so that the injury is probably determined in some early part of the latent period. Rolly in his experiments found that washing out an isolated heart with fresh blood had no effect in postponing the death of the heart, and apparently this is true. But while Rolly was unable to revive a poisoned heart it proves to be not impossible, as the following protocols show:

Dog 16. May 13, 1911. Isolated heart from normal dog perfused with Ringer's solution beat for two and a half hours at a rate of about 103. Ten drops of diphtheria toxin injected into the circulating fluid caused an immediate increase in rate to 200, after which the heart continued to beat for two hours longer at a rate of about 150.

Dog 17. May 24, 1911. Isolated heart from normal dog perfused and beating for one hour at a rate of 98. At 12.25, 1 c.c. of diphtheria toxin was added to perfusion fluid. This was followed by a marked stimulation of force and rate which rose to 112. The heart continued to beat until 3.30, when, after a period of dissociation of auricular and ventricular beats, it stopped.

Dog 18. May 25, 1911. Weight, 5.7 kg. Given 1 c.c. toxin intravenously at 11.10 A.M. At 6 P.M. carotid pressure low. At 10.30 dog was extremely ill and died at 10.55. Heart found to have stopped beating. It was removed from the body and perfused with Ringer's solution, upon which it began to beat at 11.10 and continued to beat well for one hour and ten minutes.

Dog 19. May 25, 1911. Weight, 5.9 kg. Given 1 c.c. diphtheria toxin at 11.30 A.M. At 5.45 P.M. dog was killed with ether and heart excised and perfused with Ringer's solution. From 6.45 to 10.55 it beat strongly and regularly at a rate of about 90 to 110.

Dog 20. May 25, 1911. Weight, 6.8 kg. Given 1 c.c. diphtheria toxin at 11.45 P.M. At 1 P.M. next day dog was nearly dead. Killed at 1.35 and

heart perfused. The rate was somewhat irregular, varying from 44 up to 110, but for the greater part of the next two hours it beat strongly at about 62, stopping at 4.50 P.M.

Dog 21. May 28, 1911. Weight, 7.8 kg. Given 2 c.c. of diphtheria toxin intravenously at 9.45 P.M. At 11 A.M. next morning the dog was found dead, although alive and apparently fairly well a few minutes before. Heart excised but could not be started in perfusion apparatus until 11.50. At first there was fibrillation, but this was stopped by injection of potassium chloride, after which it beat somewhat irregularly until 2.15 P.M., a period of two hours and twenty-five minutes.

Dog 22. May 30, 1911. Weight, 8.7 kg. Given 2 c.c. diphtheria toxin at 11 P.M. Next day at noon dog died; splanchnic vessels much dilated. Heart perfused at 12.15 and beat fairly well for three hours and forty minutes, becoming edematous at last. For most of the time the rate was 96 to 100, but several times a spontaneous heart-block occurred, with a 1:2 rhythm, which each time suddenly changed back to the 1:1 rhythm.

Dog 23. May 30, 1911. Weight, 8.2 kg. Given 1.5 c.c. diphtheria toxin at 11 P.M. Next day at 3.15 P.M. dog was evidently dying. Was killed with ether and heart excised and perfused. Perfusion began at 4 P.M., and heart beat with varying rate until 7.20, a period of three hours and twenty minutes.

Dog 24. June 1, 1911. Weight, 7 kg. Given 2 c.c. diphtheria toxin at midnight. At 11 A.M. next day dog was *in extremis*. Carotid pressure very low. Etherized, heart excised and perfused. It beat slowly from 11.20 to 2.25, about three hours, at a rate of about 52.

Dog 25. June 2, 1911. Weight, 7.2 kg. Given 2 c.c. diphtheria toxin at midnight. At 1.15 next day dog was very sick. Alcohol by stomach tube had no effect upon blood-pressure, which sank until the dog's death at 2 P.M. At 2.10 heart was perfused and beat normally at a rate of about 94 for three hours and thirty minutes, stopping at 5.40.

Dog 26. June 3, 1911. Weight, 8.9 kg. Given 2 c.c. diphtheria toxin at 11.15 P.M. Next morning at 9.30 dog was found dead, but not cold. Rigor beginning. At 9.45 the excised heart was perfused and beat until 10.40, at the rate of about 100, rather feebly and irregularly.

In all these hearts the beat is rather feeble as compared with that of the perfused normal heart, and fibrillation seemed very likely to occur. The note reads: "There is no comparison between the perfused normal heart and the diphtheria hearts all of which are feeble and apt to go into fibrillation." Nevertheless, although one may receive the impression that the hearts from the poisoned animals are rather weak and apt to be irregular, it is clear that

they continue to beat for several hours after they have shown every sign of failure in the body of the dying animal, if only the pressure of nutritive fluid be maintained in the coronary arteries. Indeed, the animal may be allowed to die and an hour after its death the heart can be revived and will beat for a long time. All of this seems to show fairly well that the death which occurs in the height of an attack of diphtheria is not exclusively the result of direct injury to the heart, although that may play some part in the process.

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[Thursday Afternoon, August 7]

Chairman—Professor S. G. Shattock, F.R.C.S.

SECTION III

GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY

INDEPENDENT PAPER

EXPERIMENTAL STUDIES IN TETANY

By W. G. MacCallum, M.D., Columbia University, New York

The question of the intimate nature of the convulsive symptoms which follow upon the extirpation of the parathyroid glands has long offered a field for study, but we seem to be very little nearer its solution than we were at the beginning. The matter is of especial interest since it seems to be one of the simplest examples of the way in which the organs of internal secretion act. Nevertheless, unexpected complexities appear and we are still far from its complete elucidation.

In a paper [1] before the German Pathological Society last year I was able to show that on the destruction of the parathyroid glands there arose a change in the character of the blood, such that, when it was brought into contact with the nerves of a normal animal, it would produce in them electrical hyperexcitability, so characteristic of tetany. This was proven back and forward by a great variety of experiments and controls. Animals with tetany were made to pour their blood through the leg of a normal animal or vice versa, and then in each case they were separated and the continuity of the femoral vessels, &c., established so that the leg was again supplied with the animal's own blood. Control anastomoses were made between normal animals, and finally the blood of both normal animals and animals in tetany was defibrinated and used alternately to perfuse the vessels of an isolated leg. In every instance when the nerves were bathed with tetany blood they became hyperexcitable, only to return again to their normal excitability when normal blood was again passed through the vessels.

Thus, having established the fact that the blood itself is changed in this peculiar way when the parathyroids are lost, and that it is undoubtedly responsible for the striking symptoms of tetany, it remained to attempt to discover the nature of this change. We have not been successful in this quest, but it is the object of this paper to bring forward

[1] *Mitth. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv. 941.

in outline the evidence which has been gained during the past winter, and which will be published in detail elsewhere.

We have long held the idea that a disturbance of the calcium metabolism, such that the blood and tissues are impoverished with respect to calcium, may form the basis of these symptoms, and this (*a*) is one possibility in our working hypothesis. The others are (*b*) that there may be a poison resembling an oxalate circulating with the blood and rendering inactive the calcium present there, and (*c*) that there may be a poison resembling some such substance as strychnia and causing directly a hyperexcitability of the nerves.

We have been able to approach these possibilities mainly by indirect methods as follows: If the nerves are hyperexcitable because of a poverty of calcium in the blood, the addition of parathyroid extract ought not to improve the condition since it brings practically no calcium. If this poverty of calcium is produced by an oxalate-like poison, the extract of parathyroid might annul that poison and allow the normal calcium content to reappear in time. If there is, on the other hand, a specific poison causing the hyperexcitability of the nerves, the parathyroid extract should counteract or neutralize it completely and restore the animal to normal.

The actual results are as follows: The addition of parathyroid extract to the tetany blood used in perfusing a normal leg has practically no effect, even in large doses, in lowering the excitability of the nerves. This is compatible with the idea that the blood is poor in calcium, but not with the third possibility that the blood contains a special poison. In the case of an oxalate-like substance there might not be a sufficient source for calcium in an isolated leg, so that even if the extract could neutralize that poison the result would remain obscure.

Parathyroid extract injected in large doses into the veins of animals in tetany will keep them alive longer than untreated animals and mitigates or stops the tetany, but the excitability of the peripheral nerves remains unchanged. In this respect it seems to act almost like an anæsthetic such as ether, which, affecting the ganglion cells far more than the peripheral nerves, will stop tetany instantly, while leaving the peripheral nerves hyperexcitable.

The fact that bleeding an animal in tetany and replacing the blood by an indifferent fluid will stop tetany, a fact cherished as evidence that a definite poison must be circulating in the blood, really demands another explanation. This procedure lowers greatly the excitability of the nerves and does so also in normal animals. It seems clear, then, that it acts not by merely removing a circulating poison, but by so disturbing the nutrition of the nerves that their irritability, which is most sensitive to any change in the blood, is lowered just as it would be by complete anæmia or any other injury. Were it not for the wonderful power of the body to restore the blood quickly to its normal state, the effect upon the nerves might be even more profound and lasting. The injection

of sodium oxalate into the blood will, under certain circumstances, produce a hyperexcitability like that seen in tetany. When it is added to the blood used for perfusion it does this, but unless it is used in extremely small quantities it ends by killing the nerve. Injected into the veins of a normal animal it will produce the same effect, but only when it is introduced very slowly at intervals over a long time. Otherwise it may kill the animal without having changed the excitability of the nerves. This is probably because it takes time to gradually exhaust the calcium which can be furnished by the tissues, and perhaps the same thing holds good of the latent period which follows the destruction of the parathyroids before tetany appears.

Direct analysis of the blood, which was carried out for me by Dr. Karl Vogel, shows, as Voegtlin and I[1] stated in an earlier paper, that its calcium content is greatly reduced in tetany. The administration of parathyroid extract does not affect this, for in the blood of animals kept alive by parathyroid extract for several days the amount of calcium was the same as that of dogs which had died in tetany without such treatment. This seems to support the idea that there is a direct loss of calcium, but is rather opposed to the possibility that a poison of the character of an oxalate is present, for in that case the calcium should be still recognizable, and its inactivation should be stopped by the parathyroid extract.

If a little parathyroid tissue be left behind, so that tetany fails to appear, the calcium content of the blood is that of a normal animal. Taking all these facts into consideration, it seems that, although we have ourselves tried to shake our original theory that tetany is dependent upon a disturbance of the calcium content of the blood and tissues, the evidence at the moment is rather favourable to that view. Still, rather than express any decided conclusion on such evidence, we shall continue to search for more.

[1] *Journ. Exp. Med.*, 1909, xi. 118.

XII. Die Nebenschilddrüsen.

Von

W. G. Mac Callum-New York.

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Aus den Arbeiten über die Tetanie, besonders in ihrer Beziehung zu den Nebenschilddrüsen, wie sie in den Monographien von Frankl-Hochwart und Escherich zusammengestellt sind, und aus den Arbeiten mehrerer anderer Autoren (Bircher 1911, Chantemesse und Marie 1893, Glaserfeld 1909, Landois 1908, Laguesse 1909, Leighton 1908, Rudinger 1909, Schirmer 1907, v. Verebely 1910, Wirth 1910 und Wassertrilling 1908), geht hervor, daß die Literatur über den Gegenstand hinreichend zugänglich ist.

Und doch sind einige Punkte, die zu dem wichtigen Thema in Beziehung stehen, noch nicht kritisch erwogen worden; auch sind wir seit der Arbeit von Rudinger in dieser Zeitschrift merklich weiter gekommen.

Anatomie.

Neuere Autoren haben zur Kenntnis der embryonalen Entwickelung der Nebenschilddrüsen im ganzen nur wenig beigetragen: sie haben lediglich darauf aufmerksam gemacht, daß akzessorisches Parathyreoidgewebe ein häufiges Vorkommnis ist, worauf in vielen Fällen, so bei den vergeblichen Versuchen, durch Exstirpation der Hauptdrüsen eine experimentelle Tetanie zu erzeugen, geachtet werden muß. Das betonte vor allem Erdheim in seinen Studien über die Tetanie bei der Ratte, einem Tier, dessen Thymus zahlreiche Parathyreoidgewebsinseln enthält. Hanson zeigte, daß diese Inseln beim Kaninchen entlang des cervicalen Teils der Thymus aus der Parathyreoidanlage der dritten Kiementasche entstehen, während aus der der vierten Tasche keinerlei solche Bildungen hervorgehen. Pepere fand ebenfalls eine weitläufigere Verteilung von akzessorischen Parathyreoidkörperchen in der Thymus, die ihren Ursprung aus der dritten Kiementasche nehmen und die unter Umständen die Unklarheiten in dem Ausfall des Experiments, sowie die Herkunft von Cysten und Tumoren in der Thymus erklären könnten.

Solche akzessorischen Massen finden sich beim Kaninchen, der Ratte, dem Menschen und persistieren unabhängig von der Rückbildung der Thymus, als Zellstränge oder Zellinseln, an der dorsalen Oberfläche dieser Drüse, und zwar in ihrer Kapsel oder in Form von epithelialen Kanälen zwischen den Läppchen. Michaud und Getzowa fanden vereinzelt Parathyreoidgewebe tief eingebettet in der Thyreoidea, weit entfernt von den Hauptdrüsen.

Andere Schriften, vor allem aus der Feder von Chirurgen, beschäftigen sich mit der genauen Lage der Drüsen, ihren Beziehungen zu den Blutgefäßen der Thyreoidea, zu ihren Nerven und zu anderen topographischen Orientierungspunkten, die ihre Auffindung und Schonung während Operationen an der Thyreoidea ermöglichen. So die Arbeiten von Berard und Alamartine, Delorme und Alamartine, Ginsborg und anderen. Jedoch fügen sie wenig zu den älteren Darstellungen von Chantemesse und Marie, Civalleri, Mac Callum, Kursteiner, Halsted und anderen hinzu. Bei längeren Reihen von einfachen Präparationen findet man offenbar nicht in jedem Falle vier Drüsen; allerdings mag die Schwierigkeit, sie zu erkennen, und die Unregelmäßigkeit ihrer Lagerung dabei in Betracht zu ziehen sein. In einer genaueren Zeichnung, die durch Halsted nach Mac Callums Präparationen entworfen wurde, kommt zur Darstellung, daß jeder Punkt entlang der Hinterfläche der Thyreoidea von einer oder der anderen Drüse eingenommen werden kann, während sie andererseits manchmal in dem losen Gewebe des Halses unterhalb der Thyreoidea und weit von ihr entfernt liegen können. Auch mehr als vier Drüsen sind für den Men-

schen ermittelt worden (Mac Callum, Erdheim); beim Hund fünf, sechs oder noch mehr zu finden, ist keineswegs etwas Ungewöhnliches.

Vom chirurgischen Standpunkte aus höchst wichtig ist die Kenntnis der Anatomie der Blutversorgung. Halsted und Evans stellten mittels Injektionen den Ursprung einer eigenen Parathyreoidarterie fest, die gewöhnlich aus der Arteria thyreoidea inferior oder ihren Anastomosen mit der Arteria thyreoidea superior entspringt und in die Drüse an ihrem Hilus eindringt, so daß die Parathyreoideae keine direkte Verbindung zu den Gefäßen des Bindegewebes haben, in dem die Thyreoidea eingebettet liegt, und nicht von ihnen abhängen. Seither hat Evans im einzelnen die Gefäßversorgung der Drüsen untersucht und gefunden, daß die Arterie zentral zu liegen kommt, während der venöse Abfluß in oberflächlich gelegenen, stark hervortretenden Gefäßen statthat.

Praktisch haben sich unsere Kenntnisse über den histologischen Bau dieser Drüsen seit der Darstellung Rudingers nicht geändert; nur in der Kenntnis der sekretorischen Prozesse in den Zellen ist durch Engel und die Beobachtungen von Haberfeld und Schilder bezüglich der Hypertrophie der Drüsen ein Fortschritt zu verzeichnen. Engel vermochte mit Hilfe der Methoden von Galeotti und Altmann drei Typen von Zellen zu unterscheiden: Große, helle, bläschenartige, kaum gefärbte Zellen und kleine stark färbbare Zellen, Typen, die freilich nicht allzu scharf zu trennen sind. Nach der Methode von Galeotti zeigen gewisse Zellen, offenbar die, welche man gewöhnlich als acidophile mit kleinem dunkel gefärbtem Kern erkennt, gleichmäßig runde, helleuchtende, fuchsinophile Granula, die als Sekretkörner angesehen werden; aber da dort kein Sekretgang besteht, so haben sie keine bestimmte Anordnung; die Wahrscheinlichkeit, daß es Sekretionsprodukte sind, ist durchaus nicht bestimmt, denn die Zellen bilden selten Bläschen. Die großen Zellen der ersten Gruppe haben ein retikuläres Protoplasma mit gelegentlichen Vakuolen. Sie enthalten einige grünlichgraue Granula, die in ihren Farbenreaktionen stark variieren, und sehr groß, selbst bis zu 4 μ, sein können. Rote Granula treten bisweilen auch auf. Außer den fuchsinophilen und basophilen Granula finden sich Fettkugeln, die von Erdheim und anderen beschrieben worden sind. Alle diese Gebilde existieren während des Lebens und sind Äußerungen einer funktionellen Tätigkeit.

Pepere machte ähnliche Studien an verschiedenen Tieren und betrachtete die chromophilen Zellen als die mehr aktiv funktionierenden, da er sie stark entwickelt fand in einzelnen zurückgelassenen Nebenschilddrüsen, die 18 Monate nach der Zerstörung der anderen noch bestehen blieben. Ebenso zeigen sie eine stärkere Entwicklung während der Schwangerschaft. In dem Gewebe der akzessorischen Parathyreoidinseln anderer Organe findet er konstant Fett und Glykogen, ohne jedoch eisenhaltige Granula in den Zellen nachweisen zu können.

Im Zusammenhang dieser Beschreibung ist noch zu erwähnen, daß zahlreiche Beobachter in den Nebenschilddrüsen die Bildung alveolarer Räume mit epithelialer Auskleidung und Kolloidinhalt gesehen haben.

In der Tat sind diese keineswegs selten, obwohl sie nicht oft ein gerade hervorstechendes Merkmal bilden. A priori wird man annehmen, daß die Kolloidsubstanz chemisch verschieden ist von derjenigen, die sich in der Thyreoidea oder anderswo findet, jedoch wissen wir über ihre Zusammensetzung nichts Näheres.

Haberfeld und Schilder betonen das Vorkommen von akzessorischem Parathyreoidgewebe in der Thymus von Kaninchen und finden, daß eine hochgradige kompensatorische Hypertrophie Platz greift, wenn die Hauptdrüsen entfernt werden. Dieser Prozeß, der für die Thyreoidea so einleuchtend ist, wurde für die Parathyreoideae nicht immer so ohne weiteres angenommen. Tatsächlich haben ihn manche Autoren in Frage gestellt, während andere, die wohl akzessorisches Thyreoidgewebe beobachteten, sich zu der Behauptung bestimmen ließen, daß die Parathyreoideae, die nach der Exstirpation der Thyreoidea zurückbleiben, thyreoides Gewebe produzierten. Diese Ansicht ist durchaus nicht mehr länger haltbar.

Physiologie.

Die zahlreichen Arbeiten, die im letzten Dezennium über die Physiologie der Parathyreoiddrüsen erschienen sind, haben doch nur weniges zur tatsächlichen Erkenntnis beigetragen. Sie beschränken sich auf das Studium der Rolle, die diese Drüsen in dem Gesamtstoffwechsel des Körpers und speziell im Kalkstoffwechsel spielen, und auf die unbestimmte Erkenntnis von den Korrelationen der verschiedenen Organe mit innerer Sekretion, an denen jene mitbeteiligt sind. Über ihre anatomische und funktionelle Selbständigkeit und die Abhängigkeit der experimentellen Tetanie von ihrer Zerstörung braucht seit den grundlegenden Arbeiten von Vassale und Generali der Beweis nicht mehr geführt zu werden; von ihnen sind diese Punkte, jetzt Gegenstand allgemeiner Übereinstimmung, definitiv festgelegt worden. Bei der Besprechung der ganzen Frage von der Insuffizienz der Schilddrüsen und Nebenschilddrüsen hob Jeandelize im Jahre 1900 die Unabhängigkeit beider Drüsen voneinander hervor und betonte die Bedeutung der Nebenschilddrüsen für die Tetanie, so wie wir sie heutzutage im großen ganzen anerkennen. Die meisten neueren Arbeiten, die sich mit diesem Gegenstande beschäftigen, sind somit zum größeren Teil nur Wiederholungen dieses älteren Werkes, obgleich leider nur zu oft die Resultate ihnen zugute geschrieben werden. Das mag wohl daher kommen, daß die italienischen und französischen Publikationen verhältnismäßig unzugänglich sind. Das Fazit jedenfalls aus all den Arbeiten ist die klare Erkenntnis, daß der Symptomenkomplex der Tetanie die unmittelbare Folge der Destruktion der Nebenschilddrüsen ist.

Da es sich nun aber gezeigt hat, daß ein recht kleiner Teil von Nebenschilddrüsengewebe imstande ist, ihr Auftreten zu verhüten, so ist es wahrscheinlich, daß diejenigen Fälle, bei denen die experimentelle Exstirpation aller sichtbaren Nebenschilddrüsen nicht zur Tetanie geführt hat, so aufzufassen sind, daß die in der Thymus so häufig vor-

kommenden akzessorischen Drüsen gerade bei diesen operierten Individuen vorhanden und imstande waren, die Funktion des Organs zu erfüllen. Das scheint die plausibelste Erklärung zu sein für die Schwierigkeit, eine experimentelle Tetanie z. B. beim Schaf zu erzeugen (Cadeac, Guinard, Lanz, Simpson, Mac Callum), da in den Fällen, in denen die Exstirpation eine vollständigere ist, typische Tetanie auftritt.

Ob der nämliche Symptomenkomplex der Tetanie auch bei Gegenwart von aktiv funktionierenden Nebenschilddrüsen hervorgerufen werden kann, ist unsicher, doch angesichts der anatomischen Befunde in manchen Fällen von gastrischer Tetanie, Arbeitertetanie und sogar infantiler Tetanie offenbar nicht unwahrscheinlich, da in diesen Fällen die Nebenschilddrüsen vollkommen normal befunden werden. Äußere Umstände scheinen die Tetanie häufig auszulösen. Massaglia konstatiert, daß äußerste Muskelanstrengung einen Anfall bei Tieren auslösen kann, die nur partiell parathyreoidektomiert waren, und schließt daraus, daß die Nebenschilddrüsen gewöhnlich toxische Substanzen, die beim Stoffwechsel des Muskels gebildet werden, neutralisieren. In ähnlicher Weise findet Boldyreff, daß, wenn man ein Tier in einer Temperatur von 38—40° C hält, sehr leicht Tetanie auslösbar wird, während die Eigenwärme des Tieres um 3—4° C steigt. Wird es in einen kalten Raum gebracht, so verschwinden die Symptome und die Temperatur sinkt. Daraus folgert er, daß die Wärmeregulation eine schwere Störung erleide. Es ist allerdings wahr, daß die Körpertemperatur im Verlauf eines heftigen tetanischen Anfalles steigt, aber sie sinkt wieder mit dem Nachlassen der Muskelkrämpfe; demnach scheint der Wärmeregulationsmechanismus nicht mehr gestört zu sein, als bei einem Schnelläufer, dessen Temperatur vorübergehend infolge seiner heftigen Anstrengung auch ansteigt, da in deren Verlauf die Wärmebildung so intensiv sein kann, daß der Mechanismus der Wärmeabgabe dem nicht die Wage halten kann.

Die neuen Forschungen auf dem Gebiete der Korrelationen der Organe mit innerer Sekretion haben solche komplizierten Verhältnisse enthüllt, daß es unklug wäre, irgendwelche endgültigen Schlüsse mit Beziehung auf die Funktion eines einzigen dieser Organe zu ziehen, ohne den Einfluß der anderen mitzuberücksichtigen. Nichtsdestoweniger ist es notwendig, um eine Vorstellung von der Rolle solcher Organe wie der Nebenschilddrüsen zu erhalten, erst deren Tätigkeit allein zu beobachten, und uns dabei zu vergegenwärtigen, daß wir die Ergebnisse dieser Studien wahrscheinlich zu modifizieren haben werden, sobald wir dazu übergehen, ihre Beziehungen zu den anderen Organen zu betrachten. Anstatt daher zuerst das weit umfangreichere Phänomen zu studieren, wie es durch die ziemlich planlose Zerstörung zweier oder mehrerer Organe hervorgerufen wird, was oft genug geschehen ist, sollten wir lernen, was ein Organ allein leisten kann. Es mag sein, daß manches, was gerade über diese Drüse gearbeitet worden ist, daher von geringerem Werte ist, daß in manchen Untersuchungen einem leichteren Operieren zuliebe die Thyreoidea samt den Parathyreoideae exstirpiert worden ist.

Die Tetanie und das Nervensystem.

Das Hauptinteresse bei alledem richtete sich auf die Bestimmung der Faktoren, die die charakteristischen Symptome der Tetanie erzeugen, wie sie infolge ungenügender Wirksamkeit der Nebenschilddrüsen in die Erscheinung tritt. Die meisten der älteren Autoren begnügen sich mit der Hypothese, daß das Fehlen der Nebenschilddrüsen oder ihrer Sekretion eine Art von Vergiftung hervorruft, die wahrscheinlich durch die Anhäufung einer gerade das Nervensystem schädigenden oder reizenden Substanz in den Gewebssäften bedingt wird, so daß eine Überempfindlichkeit mit der Neigung zu muskulären Spasmen daraus resultiert. Tatsächlich ist eine solche giftige Substanz im Blute nicht nachgewiesen worden, wenn auch in der Literatur einzelne vage Befunde mitgeteilt sind, die das Auftreten unbestimmter tetanischer Symptome nach Einspritzung von Tetanieblut bei einem normalen Tier betreffen (Colzi, Fano und Zanda, Rogowitsch, Sgobbo und Lamari, Gley, Biedl u. a.). Pfeiffer und Mayer wiederholten diese Versuche an Hunden mit negativen Resultaten, und Mac Callum berichtete über mehrere ähnliche Versuche an Hunden, nach manchen Richtungen variiert, um dem injizierten Blute zur Entfaltung einer gewissen Wirksamkeit Gelegenheit zu geben. Sogar nach partieller Parathyreoidektomie brachte die Injektion von Blut, das einem Hunde mit ausgeprägter heftiger Tetanie durch Verbluten entnommen war, keinerlei definitive Symptome hervor, auch wenn der Hund in weiten zeitlichen Zwischenräumen bereits Andeutungen von tetanischen Anfällen erkennen ließ, also nahe der Grenze der Parathyreoidinsuffizienz war. Das Experiment ist zu kompliziert, da es auf der Möglichkeit einer Störung der Balance beruht, die durch die Parathyreoidreste erhalten wurde. Augenscheinlich war die Dosis des Tetanieblutes, obwohl groß, doch nicht groß genug, um dieses Verhältnis entscheidend zu stören.

Pfeiffer und Mayer haben in ihren Versuchen das Serum von an Tetanie leidenden Hunden in die Blutbahn von Mäusen gebracht und fanden, daß normale Mäuse gesund blieben, partiell parathyreoidektomierte Tiere dagegen in Tetanie starben. Daraus schließen sie, daß ein zirkulierendes Toxin im Blut vorhanden sei. Die weiteren Komplikationen in diesen Versuchen, wie sie durch das artfremde Serum, durch die Benutzung von Serum anstatt von Gesamtblut und endlich durch die all diesen Versuchen gemeinsame Gefahr gegeben sind, daß nämlich die Tetanie durch Parathyreoidektomie selbst ausgelöst ist, vermindert den Wert ihrer Beweiskraft natürlich sehr.

Das weitere Studium dieser Frage von der Gegenwart eines zirkulierenden Toxins im Blut muß abhängen zunächst von einer klareren Erkenntnis des Wesens der Wirkungen und der Art der Gewebe, die dabei betroffen sind. Die Symptome sind manifest genug, und doch ist zurzeit noch nicht entschieden, ob die Störung im Gehirn, Rückenmark, den peripheren Nerven oder den Muskeln gelegen ist.

Erschöpfende Untersuchungen über diesen Gegenstand sind zurzeit noch nicht durchgeführt worden. Lanz schloß, daß der „Angriffspunkt"

des hypothetischen „Tetaniegiftes" weder in den peripheren Nerven noch im Rückenmark, noch auch in den motorischen corticalen Rindenbezirken gelegen sei. Mac Callum hatte in früheren Experimenten gefunden, daß in einer Extremität, zu der die Nerven durchschnitten wurden, keine Spasmen auftraten. Er fand relativ schwache Zuckungen in den gelähmten Extremitäten nach der Durchschneidung des Rückenmarkes, wobei die Muskeln schlaff waren. In den jüngeren Experimenten von Biedl, Falta und Rudinger aber waren solche Zuckungen mehr ausgesprochen, so daß der Zustand der hinteren Extremitäten in solchen Fällen sich von dem der intakten vorderen Extremitäten hauptsächlich im Verlust der tonischen Contraction unterschied. Die Vorderläufe eines solchen Tieres werden starr gehalten; ein Schauer kann darin gefühlt werden, häufig unterbrochen durch spasmisch-klonische Contractionen, während in den Hinterläufen die klonischen Contractionen allein auftreten. Durchschneidung des Rückenmarkes scheint daher den bei der Tetanie gesteigerten tonisierenden Einfluß des Zentralnervensystems auf die Muskeln zu beheben, während die Übertragung von Zuckungen durch Impulse aus den Vorderhornzellen möglich bleibt. Es ist klar, daß, wenn die peripheren Nerven vom Rückenmark getrennt sind, keine Impulse, was für welche immer es sein mögen, zu den Muskeln gelangen können, so daß eine entsprechende Extremität in vollkommener Ruhe verharren muß. Exstirpationen der motorischen Rindenregionen haben keine sehr klaren Wirkungen auf die tetanischen Zuckungen der Extremitäten in den Versuchen von Lanz und Mac Callum ergeben. Biedl dagegen berichtet über eine Steigerung der klonischen und tonischen Contractionen auf der paretischen Seite.

Übererregbarkeit der motorischen Nerven.

Hinsichtlich des Zustandes der peripheren Nerven während der Tetanie fand Biedl, daß ein Nerv, der während der Tetanie durchschnitten wird, rasch seine Übererregbarkeit einbüßt. Eppinger und Falta zeigten, daß nach Durchschneidung des Rückenmarks die Nerven der gelähmten Extremitäten überempfindlich sind, daß Ligatur der Femoralarterie keine Veränderung darin hervorbringt, und daß nach Durchschneidung des Ischiadicus vor der Tetanie der Nerv degeneriert, ohne daß er jemals übererregbar wird, während der Nerv, wenn er im Laufe der Tetanie durchschnitten wird, für eine Zeitlang erregbar bleibt. Falta und Rudinger bestätigen das in einer neueren Arbeit und ziehen den Schluß, daß bei Tetanie die vermehrte Erregung des peripheren Nerven ihren Grund hat „in einer abnormen Ladung des gesamten Neurons von dem trophischen Zentrum — der Ganglienzelle — aus mit einer bisher noch unbekannten, definierbaren Substanz. Es bliebe jetzt noch übrig, die Brücke zu suchen zwischen dem Übererregungszustande in den motorischen und sensiblen Ganglienzellen des Rückenmarks einerseits und des Sympathicus andererseits".

Mac Callum fand in neueren Experimenten, daß die Ligatur der

Femoralarterie allein keine Veränderung in der Übererregbarkeit des Nerven hervorruft infolge der reichlichen kollateralen Blutversorgung. Vollkommene Anämie einer Extremität bringt, wie wohl bekannt ist, in sehr kurzer Zeit Veränderungen in der Nervenerregbarkeit hervor, die zu ihrem allmählichen Verschwinden führt, oft schon nach einer halben Stunde. Im Laufe dieser Veränderung nimmt die KOZ und AOZ merklich gegenüber der KSZ und ASZ zu. Nichtsdestoweniger sinkt trotz dieser relativen Überempfindlichkeit die Erregbarkeit und ist allemal niedriger als in der Tetanie. Zur Beurteilung der Erregbarkeit der durchschnittenen peripheren Nerven ist es nötig, alle Nerven einer Extremität zu trennen oder alle Muskeln oberhalb der Stelle, wo man prüfen will, zu durchschneiden, da sonst ihre Contraction leicht die Resultate trübt. Mit solchen Vorsichtsmaßregeln fand man, daß die Nerven, wenn sie durchschnitten wurden, nachdem die Tetanie voll entwickelt war, ihre hohe Erregbarkeit mindestens 2 Tage bewahrten, dann allerdings sehr rasch alle Erregbarkeit überhaupt verloren und auf keinerlei Strom mehr antworteten. Werden sie durchschnitten nach der Entfernung der Nebenschilddrüsen, so nimmt ihre Erregbarkeit ebenso zu wie die der intakten Nerven. Diese Übererregbarkeit bleibt vorhanden bis zu ihrer Degeneration, die ein plötzliches vollkommenes Erlöschen zur Folge hat.

Daß das nicht notwendigerweise das Resultat einer Veränderung im ganzen Neuron einschließlich der Ganglienzellen in den Vorderhörnern ist, ergibt sich aus weiteren Versuchen, in denen die Arteria und Vena femoralis eines normalen Tieres mit der Carotis und Jugularis eines zweiten Tieres, bei dem eine voll ausgeprägte Tetanie besteht, verbunden wurden. Die peripheren Teile der Nerven und Muskeln waren so durchströmt vom Blute eines tetanischen Hundes, während das Rückenmark unverändert blieb. Der periphere neuromuskuläre Apparat nimmt in 1 Stunde den hohen Grad und die qualitativen Veränderungen der für die Tetanie charakteristischen Eigentümlichkeiten der Übererregbarkeit an. Vereinigt man die femoralen Blutgefäße wieder mit ihren eigenen Stümpfen und führt auf diese Weise der Extremität ihr eigenes Blut wieder zu, so kehrt die Erregbarkeit der peripheren Nerven zur Norm zurück. Werden die nämlichen Anastomosen zwischen zwei normalen Tieren geschaffen, so tritt keinerlei Veränderung in der Erregbarkeit auf.

Daraus geht hervor, daß bei der Tetanie die peripheren Nerven direkt lädiert werden, wobei es sehr wahrscheinlich bleibt, daß die nämlichen Einflüsse auf ähnlichem Wege Übererregbarkeit im Zentralnervensystem hervorrufen.

Diese Versuche scheinen auch einen weit zwingenderen Beweis für die Richtigkeit der Vermutung zu erbringen, daß eine Substanz im Blute angehäuft ist, oder wenigstens, daß nach der Zerstörung der Nebenschilddrüsen eine Veränderung darin Platz greift, die es instand setzt, im Nervensystem eine Übererregbarkeit hervorzurufen. Die Raschheit, mit der diese Übererregbarkeit auftritt, spricht für die Gegenwart irgendeiner aktiven Substanz im Blute.

Übererregbarkeit der sensiblen Nerven.

Bisher war nur dem Zustand der motorischen Nerven Beachtung geschenkt worden, aber an der Übererregbarkeit der sensiblen Nerven ist nicht weniger zu zweifeln. Hoffmann studierte 1888 dieses Phänomen und beobachtete Parästhesien und Erhöhung der sensorischen Erregbarkeit ebensowohl wie der motorischen auf mechanische und elektrische Reize hin, was ihn veranlaßte, in der Tetanie das Resultat einer Reizung der motorischen Nerven durch das Sensorium zu sehen, oder noch wahrscheinlicher eine Kombination von erhöhter sensorischer Reflextätigkeit und motorischer Erregung. Hesse, Niemeyer, Schultze und andere hatten keine Zunahme in der Reflexerregbarkeit gefunden, von der Hoffmann annahm, daß sie mit der Übererregbarkeit der Vorderhornzellen vereinbar sei. Chvostek hat 1891 auf die Erhöhung der mechanischen und elektrischen Erregbarkeit der sensiblen Nerven hingewiesen. Er vermochte diese elektrische Veränderung zu messen und zu zeigen, daß sie denselben Gesetzen hinsichtlich Öffnung und Schließung und Umdrehung des Stromes folgt wie bei den motorischen Nerven. Einfache Empfindung ist verbunden mit Parästhesien, ein Phänomen, das ebenso sich an den spezifischen Sinnen äußert: bei Reizung des Acusticus durch einen relativ schwachen Strom wird ein lauter Schall gehört. Auf diese Beobachtungen nimmt er 1909 in seiner Arbeit über die Diagnose der Tetanie abermals Bezug. Falta und Kahn erwähnen ebenso wie auch Frankl-Hochwart die bittere Geschmacksempfindung, die durch einen leichten elektrischen Reiz hervorgebracht wird, in Fällen von Tetanie.

Das sympathische Nervensystem.

Angesichts dieser weitgehenden Veränderungen am Nervensystem ist es nicht verwunderlich, auch Veränderungen des sympathischen Systems zu finden. Ibrahim erwähnt Fälle von kindlicher Tetanie, in denen Spasmen von After und Blase mit Stuhl- und Urinverhaltung vorhanden waren, einen anderen Fall mit Ungleichheit der Pupillen, die er einem Spasmus des Dilatator pupillae zuschreibt. Ferner führt er vasomotorische Störungen an, in deren Gefolge Ödeme an den Beinen usw. auftraten, und Herzstillstand, den er als Herztetanie analog dem Laryngospasmus auffaßt.

Falta und Kahn haben die Fragen der Beziehungen der Tetanie zum vegetativen System in ihre neueste Arbeit aufgenommen und finden, nach dem Studium ihres klinischen Materials, manche Äußerung einer Übererregbarkeit des Sympathicus. Beide, Adrenalin und Pilocarpin, zeigen eine sehr gesteigerte Wirksamkeit bei Tetanischen, was seine Ursache hat in der gesteigerten Empfindlichkeit des neuromuskulären Komplexes für sympathicotrope Reize. Symptome wie Pupillenerweiterung, Tränen- und Speichelfluß sowie Schweißsekretion sind zweifelhaft. So auch die Veränderungen im Typus der Respiration

und die kardiale Tetanie. Aber Gefäßstörungen sind gewöhnlich vorhanden, außerdem finden sich Symptome von seiten des ganzen Digestionstractus: Erbrechen, Diarrhöen, Magenkrämpfe usw., die als Veränderungen in der Tätigkeit des Sympathicus angesehen werden können.

Unsere Erkenntnis von dem wahren Wesen der Parathyreoidinsuffizienz auf das Nervensystem ist zurzeit noch recht unvollständig, obwohl der Weg für ausgedehntere Untersuchungen gebahnt scheint. So vage auch unsere Vorstellungen über die Art des Ablaufs dieser Störungen sind, so beginnen wir uns trotzdem dem Problem zu nähern, wenn wir sehen, daß das zirkulierende Blut eine bestimmte Substanz enthält, die eine Nervenübererregbarkeit hervorrufen kann oder wenigstens so verändert ist, daß daher dieser Effekt rührt. Weitere Untersuchungen des Blutes mögen von Erfolg in der Lösung dieser Frage sein, was deshalb von so großer Tragweite ist, weil wahrscheinlich damit ein Paradigma gegeben ist, mittels dessen ähnliche Fragen in Beziehung zu verwandten Drüsen in Angriff genommen werden können.

Der Stoffwechsel in der Tetanie.

Die Stoffwechseluntersuchungen nach Parathyreoidektomie haben spärliche und zum Teil widersprechende Resultate gehabt, was wohl den verschiedenen in Anwendung gezogenen Methoden zur Last zu legen ist. Dazu kommt, daß es nicht leicht ist, zwischen den Veränderungen, die auf die angestrengte Muskeltätigkeit zu beziehen sind, und solchen, die das Resultat des Verlustes der Funktion der Nebenschilddrüsen sind, zu entscheiden.

Das ist vielleicht ganz besonders der Fall hinsichtlich der Stickstoffuntersuchung. Mac Callum und Voegtlin, Berkeley und Beebe, Cooke und Jacobsohn fanden während des tetanischen Anfalls die Ammoniakmenge in Blut und Urin erheblich gesteigert, wohingegen in einer neueren Arbeit Carlson und Jacobsohn mit anderen Methoden keine wesentliche Zunahme feststellten. Ebenso findet Greenwald den Ammoniakstickstoff im Verhältnis zum Gesamtstickstoff wenig, wenn überhaupt vermehrt, und die Konzentration des Blutes an Ammoniak nicht höher als im Blut normaler Tiere. Medwedew auf der anderen Seite fand das Ammoniak im Blut bei der Tetanie vermehrt und erklärt diesen Umstand mit dem Abbau irgendeiner Substanz, wobei Ammoniak frei werde; der thyreoparathyreoide Apparat liefere ein Ferment oder eine Desamidase.

Einige dieser Autoren (Berkeley und Beebe, Jacobsohn) dachten sogar, daß das Ammoniak in ursächlicher Beziehung zur Tetanie stünde und betrachteten es als Index für die Gegenwart einer giftigen Substanz oder selbst als das Gift; Jacobsohn fand diese Annahme bestätigt durch die Beobachtung, daß die nämliche Menge Ammoniak erforderlich ist, um Konvulsionen zu erzeugen, die sich beim Tiere während eines tetanischen Anfalls im Blute vorfindet. Später aber zogen sie diesen

Gedanken wieder zurück, nachdem sie gefunden hatten, daß Ammoniak bei einem Tiere nach Durchschneidung des Rückenmarks Krämpfe in den gelähmten Extremitäten erzeugt, während sie keine derartigen Konvulsionen am Tetanichund nach der Durchschneidung fanden. Voegtlin und Mac Callum zeigten ferner, daß Ammoniaksalze die Erregbarkeit der motorischen Nerven nicht erhöhten und daß ihre Injektion gerade im Gegenteil die Symptome der Tetanie herabsetzten.

Frouin ist der Ansicht, daß Carbaminsäure im Blute zirkuliert und im Urin in vermehrter Menge ausgeschieden wird, und daß sie als Ursache des Zustandes anzusprechen sei. Aber Greenwald erhebt ernsten Zweifel dagegen, da die Wahrscheinlichkeit der Gegenwart von Carbaminsäure in einem nicht stark alkalischen Urin noch sehr zur Diskussion stehe. Die ausgedehntesten neueren Untersuchungen über Veränderungen im Stoffwechsel nach Parathyreoidektomie stammen von Cook und Greenwald. Cooks Resultate sind die folgenden: Nach vollständiger Parathyreoidektomie mit partieller Thyreoidektomie findet sich eine bemerkenswerte und konstante Zunahme des Stickstoffs im Urin und im speziellen der Ammoniakkomponente. Das Kreatinin und die Aminosäuren bleiben praktisch unverändert, während vielleicht eine geringe Zunahme in dem nichtspezifizierten Stickstoff, die wie die Ammoniakzunahme auf Kosten des Harnstoffes besteht, zu finden ist. Bei zweien der Tiere, der einzigen, bei denen nach dieser Richtung untersucht wurde, erschien Milchsäure im Urin gleichzeitig mit dem Auftreten des tetanischen Anfalls und mit dem Anwachsen der Stickstoff- und Ammoniakausscheidung. Ein Tier, bei dem keine Tetanie auftrat, starb mit erheblichen Ernährungsstörungen, die die Folge der Operation waren, wobei der Urin dieselbe Zunahme der Stickstoff- und Ammoniakfraktion aufwies, die die Hunde mit richtiger Tetanie charakterisierte. Die Gegenwart von Milchsäure im Urin hat mehrere Erklärungen gefunden (exzessive Muskelanstrengung, Erschöpfung der Leberfunktion oder die Unfähigkeit, einen Überschuß von Ammoniak im Blut zu neutralisieren). Wahrscheinlich hängt die vermehrte Ausscheidung von Magnesia und anderen Basen im Urin damit zusammen. Eine andere Vermutung ist die, daß die Milchsäure im Urin infolge des Daniederliegens der oxydativen Prozesse während des tetanischen Anfalls erscheint; keinesfalls ist der Ausscheidung der Milchsäure irgendeine ätiologische Bedeutung beizumessen. Greenwald kritisiert Cooks Resultate mit Rücksicht auf die hohen Werte der Ammoniakausscheidung und bezieht diese einmal darauf, daß die Tiere im Hungerzustand gewesen seien, zum andern setzt er aus, daß ihr Urin durch Katheterismus entleert wurde und dadurch einer ammoniakalischen Gärung leicht zugänglich war. Greenwalds eigene Resultate, die er von Stoffwechseluntersuchungen am Hunde erhielt, bei konstanter Diät ohne Anwendung des Katheters und nach alleiniger Exstirpation der Nebenschilddrüsen sind die folgenden:

1. Die Stickstoffausscheidung war erst nach dem Auftreten der Tetanie gesteigert.

2. Die Harnstoffkomponente waren im Verhältnis zur Menge des Gesamtstickstoffs herabgesetzt.
3. Die Ammoniakkomponente war verhältnismäßig nur wenig, wenn überhaupt gesteigert.
4. Die Konzentration des Blutes an Ammoniak war normal.
5. Die Kreatininausscheidung war ungefähr dieselbe wie vor der Operation oder wenig herabgesetzt.
6. Das Kreatin im Urin war sehr erheblich vermehrt.
7. Der Purinstickstoff im Urin war vermehrt.
8. Die nicht spezifizierbare Stickstoffkomponente im Urin war gesteigert.
9. Die Ausscheidung von Schwefel im Urin war vermehrt. Die Zunahme beruhte hauptsächlich auf der Zunahme der anorganischen Sulfate und des neutralen Schwefels.
10. In den ersten paar Tagen nach der Exstirpation der Nebenschilddrüsen war die Phosphorausscheidung sehr deutlich vermindert. Dagegen trat nach dem Erscheinen der tetanischen Symptome eine Steigerung der Ausfuhr ein.

Die vermehrte Ausscheidung der unbekannten stickstoffhaltigen Bestandteile mag an der verminderten Leistungsfähigkeit der Leber liegen. Immerhin bilden die Versuche keine Stütze für die Annahme, daß die Tetanie als Folge der Parathyreoidektomie mit einer Vergiftung durch Ammoniak oder durch Carbaminsäure etwas zu tun habe.

Die verminderte Ausscheidung des Phosphors ist die auffallendste bisher beobachtete Änderung im Stoffwechsel des Parathyreoidektomierten. Wahrscheinlich wird eine genaue Erforschung der Form, in der der Phosphor retiniert wird, die Funktion der Nebenschilddrüsen einigermaßen beleuchten.

Falta und Kahn haben den Aminosäurestickstoff, den Polypeptidstickstoff und den Ammoniakstickstoff bei der Tetanie studiert und finden, daß im akuten tetanischen Anfall die Ausscheidung des Ammoniaks relativ und absolut vermehrt, hingegen die Aminosäurefraktion normal ist. Der Peptidstickstoff ist sehr vermehrt, aber wird so gut wie nicht beeinflußt durch Verabreichung von Glykokoll. Die Autoren unterscheiden hier scharf gegenüber dem Zustand bei nekrotisierenden Prozessen in der Leber, bei denen gerade der Aminosäurenstickstoff vermehrt ist.

Diese Untersuchungen geben uns nur wenig oder gar keinen Aufschluß über das Wesen der Stoffwechselveränderungen, die charakteristisch für die Tetanie sind; es erscheint zweifelhaft, ob überhaupt die Untersuchung der Exkrete hier mehr Klarheit bringen kann als bei den Vorgängen, wie sie das Diphtherie- oder Tetanustoxin erzeugt.

Anorganische Substanzen, Calcium.

Die Veränderungen im Stoffwechsel der anorganischen Bestandteile haben sich als lehrreich erwiesen, obgleich sie vielleicht nur der Aus-

druck von untergeordneten Störungen sind. Hauptsächlich auf das Calcium wurde die Aufmerksamkeit gelenkt, wohl einmal deswegen, weil es therapeutische Anwendung fand, zum andern, weil im Verlaufe einer chronischen Tetanie im Ossifikationsprozeß Störungen auftraten. Aber Magnesium, Natrium und Kalium sind ebenso gewertet worden, trotzdem ihre Bedeutung noch durchaus nicht hinlänglich klar ist. Die ältere Literatur, die in den Arbeiten von Gregor, Sabbatani, Loeb, Roncoroni, Stoeltzner, Bogen, Quest, Czybulski, Silvestri, Netter, v. Pirquet, Parhon und Ureche, Mac Callum und Voegtlin und anderen enthalten ist, findet sich bei Rudinger und in allen Monographien des Gegenstandes.

Die Beobachtungen von Loeb, Sabbatani, Quest, Roncoroni und anderen betreffen, wie bekannt sein dürfte, das Verhältnis des Calciums zur Nervenerregbarkeit. Stoeltzner und andere gaben dem Gedanken Ausdruck, daß die infantile Tetanie einer Calciumvergiftung zuzuschreiben sei. Bogen, Czybulsky, Quest und Netter bestritten das, und während Netter Calciumsalze mit gutem Resultate solchen Kindern verabreichte, fand Quest, daß ihr Gehirn relativ calciumarm war und daß man bei Hunden eine Nervenübererregbarkeit durch eine länger fortgesetzte calciumarme Ernährung erzielen konnte. Mac Callum und Voegtlin zeigten, daß es möglich ist, die Symptome der Tetanie bei parathyreoidektomierten Hunden durch intravenöse Injektion relativ großer Dosen von Calcium zu unterbrechen und daß es sich dabei um eine fast momentane Hilfe handelt. Sie fanden in Analysen von Blut und Gehirn, daß bei der Tetanie das Calcium darin abgenommen hatte, während seine Ausscheidung in Urin und Faeces gleichzeitig zunahm. Deshalb meinten sie, es handle sich bei der Tetanie um eine Störung in der Zusammensetzung der Gewebsflüssigkeit, welch letztere normalerweise durch die Nebenschilddrüse geregelt würde, so daß der Wegfall ihrer Sekretion die Störung des Gleichgewichts zwischen den mineralischen Bestandteilen zur Folge hätte. Möglicherweise tritt eine giftige Substanz von Säurecharakter dabei auf. Denn ein ausgiebiger Aderlaß mit Ersatz des Blutes durch Salzlösung behebt solche Tetanie. Ein aktives Gift ist bisher nicht gefunden worden, wohl aber seine Wirkung, die darin bestehen dürfte, daß eine Drainage von Calciumsalzen aus den Geweben heraus stattfindet.

Von der Verarmung an diesen rührt die Nervenübererregbarkeit sowohl als die Tetanie her.

Früher neigten die Autoren zu der Ansicht, daß die günstige therapeutische Wirkung der intravenösen Calciuminjektion auf den Ersatz des Calciums zu beziehen ist, wodurch den Nervenzellen die normale Funktion zurückgegeben wird.

Berkeley und Beebe bestätigten diese Resultate, schlossen aber, da Strontium- und Magnesiuminjektionen ähnliche Wirkungen entfalten, daß das Calcium sich wie ein Sedativum verhalte. Auch sie sprechen von einem hypothetischen Gift, wahrscheinlich einem Produkt des abnormen Stickstoffwechsels, und erwähnen, daß Ammoniak und Xanthin

gewisse ähnliche Symptome hervorrufen, die gerade so günstig durch Calcium beeinflußt werden. Einige, wie z. B. Cooke, finden den Calcium- und Magnesiumgehalt des Gehirns bei der Tetanie unverändert, finden aber in den Exkreten eine vermehrte Magnesium-, nicht Calciumausscheidung. Cooke allerdings vernachlässigte praktisch die Faeces bei seinen Untersuchungen. Wenn man unter Berücksichtigung dieser Tatsachen seine Tabellen betrachtet, so wird die Zunahme der Calciumausscheidung auffallender als die der Magnesiumausscheidung.

Seit der Zeit sind viele Arbeiten erschienen mit Rücksicht auf diese Frage, die zum Teil die kindliche Tetanie behandeln, zum Teil spezieller sich mit Wachstum und Ossifikation beschäftigen, oder solche, die die nervöse Erregbarkeit bei der experimentellen Tetanie untersuchen. Was die Beziehung des Calciums zu der kindlichen Tetanie angeht, so gehen die Meinungen hier sehr weit auseinander. Finkelstein wies 1902 darauf hin, daß bei Säuglingen einseitige Ernährung mit Kuhmilch leicht eine Spasmophilie erzeuge. Daß bei künstlich ernährten Kindern diese Disposition zu berücksichtigen sei, wurde allgemein anerkannt. Welcher Bestandteil der Kuhmilch aber dafür verantwortlich gemacht werden sollte, war weniger klar. Stoeltzner bestand in mehreren Arbeiten darauf, daß der hohe Calciumgehalt der Kuhmilch die Schuld trage, und stellte sogar fest, daß die nervöse Übererregbarkeit manchmal durch Calciumchloride gesteigert werden kann. Die Störung der Darmtätigkeit ist es, die die Ausscheidung des Calciums verhindert und die „Kalkstauung" verursacht, die durch eine Calciumvergiftung selbst einen plötzlichen Herztod hervorrufen kann. Stoeltzner meint, daß eine gewisse Kalkkonzentration zu der normalen Erregbarkeit der Nerven gehört, daß aber mit Zunehmen der Konzentration die Erregbarkeit ein Maximum erreicht, danach unter die Norm herabgesetzt wird. Wenn dann die Kalkkonzentration in den Geweben diesen Punkt eben erreicht hat, bei dem die Erregbarkeit wieder herabgesetzt wird, so hat die Beschränkung des Calciumgehalts den rückläufigen Weg wieder durch das Stadium der maximalen Übererregbarkeit hindurch zur Folge. Er konstatierte bei der Spasmophilie eine Zunahme des Calciumgehalts der Muskeln und ist der Ansicht, daß diese Disposition mit der Nebenschilddrüse nichts zu tun habe.

Niemand hat die Ansicht Stoeltzners bestätigt. Risel fand, daß nach der Calciumdarreichung in einigen Fällen die elektrische Erregbarkeit zunahm, daß aber 4 mal so oft die Erregbarkeit stark herabgesetzt sei. Bei der Spasmophilie ist die elektrische Erregbarkeit, wie sie in der KOZ und AOZ zum Ausdruck kommt, vermindert nach der Calciumgabe; die anderen Symptome gehen parallel. Klinische Versuche zeigen, daß Calcium nicht den Effekt hat, den Kuhmilch haben soll. Schabad zeigt, daß tatsächlich eine Entkalkung statthat bei der Ernährung mit Kuhmilch: bei Verfütterung von reiner Kuhmilch nämlich werden nur etwa 30 Proz. Calcium resorbiert und retiniert im Gegensatz zu 70 Proz. bei Ernährung mit Frauenmilch. Neurath zeigt in ähnlicher Weise, daß, trotzdem die Kuhmilch, verglichen mit der Frauen-

milch, einen so hohen Prozentsatz Calcium aufweist, die Aufnahme des Kalks damit nicht parallel geht, sondern daß die im Urin ausgeschiedene Menge (die man als Indikator ansehen kann) beim natürlich ernährten Kind 3- bis 16 mal größer ist als beim künstlich ernährten. Bei der Tetanie, vereint mit Rachitis, sieht Schabad eine günstige Beeinflussung der elektrischen Erregbarkeit in der Darreichung von Phosphorlebertran und zeigt, daß diese Hand in Hand geht mit der Besserung in der Kalkretention, so daß wahrscheinlich die Störung der Phosphorausscheidung die primäre ist. Er stellt fest, daß die Zuführung von Calciumsalzen den tetanischen Zustand nicht verschlechtert und hält die Ansicht Stoeltzners für unbegründet. Longo, der im Blute von tetanischen Kindern normale Calciumverhältnisse findet, schließt daraus, daß hier keinerlei Beziehungen bestehen, und hält es für wahrscheinlich, daß Kuhmilch auf Grund des Abbaues gewisser Albumine schädlich ist. Seit der Publikation der Monographie Rudingers erschien eine Anzahl von Arbeiten über die Beziehung des Calciums zu der Tetanie der Kinder. Cohn schließt aus einer Untersuchung über Calcium-, Phosphor- und Stickstoffgehalt des Gehirns tetanischer Kinder, daß das Calcium in normaler Menge vorhanden sei, daß deshalb keinerlei Grund für die Annahme einer Calciumverarmung der Gewebe vorliege. Aschenheim hebt hervor, daß nicht so sehr die absolute Calciummenge, die das Gehirn eines Kindes enthält, als vielmehr das Verhältnis der Erdalkalien zu den Alkalien von Wichtigkeit sei. Die Änderung dieses Verhältnisses bei der Substanz des Zentralnervensystems, die zum Anwachsen des Quotienten $\frac{\text{Alkalien}}{\text{Erdalkalien}}$ führt, ergibt den Symptomenkomplex der Tetanie. Das kann der Fall sein, wenn auch die Werte für Calcium und Magnesium völlig normal sein mögen. Durch alle Arbeiten von Loeb und anderen, die die Salzwirkungen in ihren Beziehungen zu der neuromuskulären Erregbarkeit studiert haben, ist dieser Gedanke gestützt und muß von denjenigen, die künftighin Untersuchungen über die chemischen Veränderungen bei der Tetanie machen, berücksichtigt werden.

Die Vernachlässigung solcher Verhältnisse stellt den wunden Punkt in allen früheren Arbeiten dar, die eine Substanz allein, z. B. das Calcium, berücksichtigt haben. Cattaneo fand weit weniger Calcium als normal im Blut von Kindern bei der Tetanie, auch eine viel geringere Aufnahme von Kalk, als verabreicht worden war. Alle seine Resultate tragen zu der Ansicht bei, als ob es sich bei der Spasmophilie um eine Hypocalcifikation des Organismus handle, oder besser um eine Alteration im Verhältnis der Salze, die von der Insuffizienz des thyreoparathyreoiden Apparates abhängig ist. Neurath, der Wrights Methode der Calciumbestimmung anwendet, fand, daß bei gesunden Kindern der höchste Calciumwert im Blute in den ersten Lebenstagen vorhanden ist; später nimmt er ab. Ferner stellt er fest, daß er höher ist bei Brustkindern als bei künstlich ernährten Säuglingen. Eine künstliche Kalkanreicherung der Nahrung führt nicht zu einer prompten Kalk-

zunahme im Blut, ja sogar zuweilen zu einer Abnahme. Im Tierexperiment führt starke Kalkanreicherung der Nahrung zu einem geringen Ansteigen im Calciumgehalt des Blutes. An Tetaniekindern findet sich eine relative „Armut des Blutes an oxalatfällbaren Kalkverbindungen"; das Experiment lehrt das nämliche. Die Nebenschilddrüsen haben eine fördernde Wirkung auf den Kalkstoffwechsel, und es scheint aus diesen Analogien klar hervorzugehen, daß es sich bei der kindlichen Tetanie um eine Erkrankung der Nebenschilddrüsen handeln soll.

Rosenstern beschreibt in seiner ausgezeichneten Darstellung des Gegenstandes seine eigenen Versuche über die Wirkung des Calciums auf spasmophile Säuglinge unter der Kontrolle der elektrischen Erregbarkeit (AOZ und KOZ) und findet, daß die besondere Übererregbarkeit unmittelbar zum Verschwinden gebracht wird unter der Darreichung des Kalks, und daß sie langsam zurückkehrt mit dem Erlöschen der Wirkung. Das Facialisphänomen verschwindet ebenfalls unter der Behandlung. Natrium hat deutlich den gegensätzlichen Effekt und erhöht die Erregbarkeit unter Fiebererscheinung und Auslösung eines Laryngospasmus. Parhon und Dumitresco geben zu, daß Calcium zwar eine sedative Wirkung hat und das Zentralnervensystem, wenn es quantitativ entzogen wird, überempfindlich macht, daß nichtsdestoweniger aber durch seine Abnahme allein keine Konvulsionen hervorgerufen werden können. Pexa setzte, nachdem er die elektrische Erregbarkeit an spasmophilen Kindern geprüft hatte, einen Hund 11 Wochen lang auf kalkfreie Kost, bis er schwach und apathisch wurde. Dabei blieb aber die elektrische Erregbarkeit seiner Nerven normal, ja sie war im Gegensatz zu den Befunden Quests eher herabgesetzt. Das Gehirn des Hundes enthielt nur ungefähr ein Viertel der Norm an Calcium.

Reiß bringt eine wichtige und genaue Studie über die elektrischen Reaktionen bei der Spasmophilie, in der der Einfluß der anorganischen Substanzen geprüft wird. Er stellt fest, daß die Erregbarkeit für die Kathodenöffnung größer ist als für die Kathodenschließung, daß dasselbe gilt für die Anodenöffnung und Anodenschließung, welch letztere relativ und absolut sinken könne, eine Behauptung, die allerdings noch fraglich erscheint, soweit die Anodenschließung in Betracht kommt. Ausgehend von der Beobachtung, daß ein Froschmuskelnierenpräparat in eine der beiden Lösungen, entweder in eine Natrium- oder in eine Calciumlösung gebracht, seine Erregbarkeit verliert, sie dagegen wieder gewinnt, wenn es in die andere gebracht wird, und von anderen Beobachtungen über die antagonistische Wirkung der anorganischen Substanzen konstruiert er eine Formel, die das Verhältnis der einzelnen folgendermaßen zum Ausdruck bringt:

Erregend	Hemmend
Li Na	Mg Ca NH_4 K

Daraus geht hervor, daß Calcium und Magnesium in der Mitte stehen zwischen hemmend und erregend. Jedoch steht das Calcium näher bei Ammoniak und Kalium, und verhält sich gegensätzlich zu Lithium und Natrium. Auf der anderen Seite ist es nicht möglich, einen Muskel, der durch Eintauchen in eine Ammoniak- oder Kaliumlösung unerregbar gemacht ist, durch Eintauchen in eine Kalklösung wieder zu beleben. Die Beobachtung, daß eine Abnahme des Calciums im Körper zu einer Steigerung der Erregbarkeit führt, wird durch die Vorstellung erklärt, daß das Calcium einem Reiter auf der Wage gleicht, dessen Verschiebung zu seinen Ungunsten dem Lithium und Natrium das Übergewicht gibt.

Reiß setzt auseinander, daß es möglich ist, die Polwirkung in einem Froschnervenmuskelpräparat durch gewisse Salze umzukehren, und daß Calcium die Kathode in Schließung und die Anode in Öffnung bevorzugt. Calcium wirkt balanzierend. Wenn es abnimmt, so werden die ASZ und KOZ verstärkt durch Ammoniak und Kalium. Von allen Kationen ist das Calcium das einzige, das gleichzeitig die Erregbarkeit herabsetzt und die Polwirkung verändert, und deshalb das einzige Salz, dessen Abnahme die Symptome der Tetanie zur Folge hat, mit Steigerung der Erregbarkeit und der Begünstigung der KOZ. Anodenüberempfindlichkeit ist nicht erklärt durch Kalkmangel; es ist nicht bekannt, ob Anoden- oder Kathodenüberempfindlichkeit einander ausschließen, oder ob beide zusammen bestehen.

In einer Tabelle drückt er diese Beziehungen folgendermaßen aus:

Normale Polwirkung		Polumkehr	
Li Na	Ca	Mg	NH_4 K

Calcium, findet er, steigert die Fähigkeit der Gewebe, sich an elektrische Ströme anzupassen und relativ unempfindlich dagegen zu werden — ein Gegenteil des Bechterewschen Symptoms, das besonders deutlich wird durch gesteigerte Empfindlichkeit der AOZ bei verlängerter Reizung.

Diese Resultate von Reiß scheinen äußerst wichtig in Verbindung mit dem Phänomen der Tetanie selbst, obwohl sie kein Licht auf die Änderungen in der AOZ werfen. Es wäre wünschenswert, daß sie zu weiteren Beweisen und Bestätigungen anregten. Sie haben in gewisser Hinsicht Beziehungen zu der Ansicht Aschenheims, der ähnliche Relationen zwischen den anorganischen Substanzen feststellt.

Silvestri und Montorsi nahmen eine kritische Stellung zu all den Arbeiten, die dem Calcium eine wichtige Rolle bei der Tetanie zuschreiben, und neigen insbesondere zu der Ansicht, daß die Calciumstörung eine sekundäre sei. Nach ihnen scheint, mit Rücksicht auf die Arbeit von Mac Callum und Voegtlin, die Calciumbestimmung von geringem Werte zu sein, sofern sie nicht über lange Zeit hin an einem Tier durchgeführt wird, und daß es unmöglich ist, zu glauben, daß Calcium solch eine Rolle in den Geweben spielt, noch daß sein

einfacher Ersatz das Stoffwechselgleichgewicht wieder herstellen könne; — eher scheint es ihnen plausibel, daß das Calcium auf die toxischen Produkte des intermediären Stoffwechsels nach der Parathyreoidektomie wirkt.

Voegtlin und Mac Callum, die die Frage nochmals untersucht haben, geben zu, daß die Bestimmungen der Calciumwerte in den Exkreten kritisiert werden können, halten aber an der Wichtigkeit der Befunde einer Abnahme der Calciumwerte im Blut und Gehirn von tetanischen Tieren fest. Sie finden, daß die Verabreichung von Calcium, Strontium und Magnesium die elektrische Erregbarkeit der motorischen Nerven beim normalen und tetanischen Tier herabsetzt bis zu solchem Grade, daß das wirksame Agens, das in den Muskeln Zuckungen erzeugt, welcher Natur es auch immer sein mag, diese Wirkung durch die betäubten Nerven hindurch nicht mehr zur Geltung zu bringen vermag. In diesem Sinne haben Berkeley, Beebe u. a. festgestellt, daß Calcium, Strontium, Magnesium in den großen Dosen, die prompt die tetanischen Symptome beheben, direkt auf die nervöse Struktur wirken und sie unfähig dazu machen, die Impulse zu leiten, durch die muskuläre Zuckungen hervorgebracht werden. Die Heilung der Tetanie durch Verabreichung dieser Substanzen bringt damit kein Licht in das Wesen der Krankheit, die zwar, doch auf die Entfernung des Calciums aus den Zellen mit einem gewissen Recht bezogen werden kann. Aber es erscheint praktisch unmöglich, das Calcium in solcher Form einzuführen, daß das, was aus den Zellen entfernt wurde, genau wieder ersetzt wird, ohne seine Grenze zu überschreiten und die nervöse Erregbarkeit abzustumpfen durch eine exzessive Wirksamkeit des Calciums.

Mit Kalium und Natrium sind die Resultate weniger bestimmte. Kalium scheint die Symptome der Tetanie zu steigern. Natriumchlorid in molekularer Lösung bringt, in sehr großen Quantitäten eingespritzt, die Symptome ohne erhebliche Herabsetzung der elektrischen Erregbarkeit zum Stehen, eine Tatsache, die vielleicht erklärt werden mag als der Effekt einer extremen Diurese, durch diese Injektion bedingt, da ein ähnliches Resultat mit Glykose erhalten wurde.

Xanthin und Ammoniak vermehren die elektrische Nervenerregbarkeit nicht und können nicht als aktive Faktoren bei der Erzeugung der Tetanie angesehen werden. Mac Callum und Voegtlin wiederholen ihre frühere Schlußfolgerung, daß die Symptome durch irgendeine giftige Substanz hervorgerufen zu werden scheinen, die im Blute kreist und die sich darin äußert, daß sie den Geweben Kalk entzieht.

Die therapeutische Darreichung von Calciumsalzen bei der Tetanie erweckte das allgemeine Interesse, wenn auch die Resultate in der Hand der verschiedenen Kliniker nicht eindeutig waren. Netter berichtete 1907 über gute Resultate bei der infantilen Tetanie nach Calciummedikation. Arthus und Schafermann gelang es, das Leben von Kaninchen durch Calciumchlorid zu verlängern. Curschmann sah von Calciumlactat günstige Beeinflussung der Tetanie, und ähnliche

Erfahrungen machte Kinnicutt in einem Falle von gastrischer Tetanie, die allerdings trotzdem tödlich endete. Frouin, der die Tetanie ätiologisch der Gegenwart von Carbaminsäure im Blute zuschrieb, fand, daß Calcium die Wirkung dieser Säure neutralisierte. Jovane und Vaglio, die zwar die Theorie der Calciumverarmung in ihrer ätiologischen Bedeutung für die Tetanie kritisierten und auf die Unstimmigkeit der verschiedenen Untersuchungen hinwiesen, fanden, daß die durch die Parathyreoidektomie gesteigerte elektrische Erregbarkeit sowohl durch Calcium als Magnesium herabgesetzt werden konnte. Falta und Kahn erreichen eine solche Herabsetzung der Erregbarkeit nicht, wenn sie ihren Patienten das Calcium per os verabreichten.

So fehlt es auch nicht an Unstimmigkeit über den Effekt des Calciums, wenngleich alle, die die experimentelle Tetanie studieren, zugeben, daß die Injektion von Calciumsalzen einen prompten Stillstand der Symptome bewirkt. Vielleicht ist die Verschiedenheit der Art der Darreichung schuld, vielleicht auch liegt der Grund in einer Verschiedenheit des Zustandekommens der Tetanie bei der infantilen Spasmophilie und der gastrischen Form der Tetanie.

Morel unterscheidet scharf zwischen der Tetanie als äußerlichem Symptom und der Kachexie, die die Äußerung einer Vergiftung und ihr wesentliches Symptom ist. Die Calciumverarmung hält er für zu unbedeutend, als daß sie imstande wäre, solche Resultate zu machen, insbesondere wenn wir die äußerste Calciumverarmung bedenken beim Diabetes, wo sie zu keinen Spasmen führt. Vielmehr scheinen die Symptome die Wirkung einer Art von Acidosis zu sein. Ferner findet er, daß traumatische Verletzung von Knochen zwar die Tetanie aufhebt oder sie verhütet, ohne Rücksicht auf ihre Art oder Intensität, daß aber diese Unterdrückung der Tetanie in keiner Weise die sonstigen Effekte der Parathyreoidektomie beeinflußt, sondern sie in ihrer ganzen Schwere bestehen läßt, so daß das Tier unter Kachexie und großem Gewichtsverlust nach 10 Tagen zugrunde geht. Er sieht die Wirkung der Parathyreoidektomie als eine Autointoxikation an, deren gewöhnlichstes Symptom, obgleich nur nebensächlich, die Tetanie ist.

Vielleicht der eifrigste Verteidiger von der Lehre der Abhängigkeit der Veränderungen bei Tetanie von Alterationen im Calciumstoffwechsel ist Erdheim, dessen so bewundernswerte Arbeit über chronische Tetanie wohl allbekannt sein dürfte. Er zeigt in zweifelsfreien Untersuchungen, daß die Exstirpation der Nebenschilddrüsen außerordentliche trophische Störungen mit sich bringt, die die epidermalen Gewebsbildungen affizieren und ebenso die Knochen in ihrem Verkalkungsprozeß stören. Hauptsächlich diesem letzteren war seine Aufmerksamkeit zugewandt. Die Exstirpation der Nebenschilddrüsen liefert einen morphologischen Beweis für die Störung des Kalkstoffwechsels, indem nämlich an den im Wachstum befindlichen Schneidezähnen von Ratten das Dentin nicht mehr verkalkt wird und die Zähne brüchig werden. Fleischmann macht dieselbe Beobachtung bei der Rachitis und der chronischen Tetanie.

Nach der Parathyreoidektomie geht die Knochenbildung nur langsam und unvollkommen vor sich. Das neugebildete Gewebe ist ein osteoides ohne Verkalkung und kein echter Knochen. Der Abbau geschieht relativ langsam, wobei dann Reste der Rinde durch osteoides Gewebe eingeschlossen werden. Bei der Heilung von Frakturen, bei denen normalerweise die Knochenenden provisorisch durch einen unvollständig verkalkten Knochencallus verbunden sind, gelten andere Verhältnisse für die Tetanie. Hier bleiben die Knochenenden durch einen Knorpelcallus weit getrennt, während der neue Callus schließlich durch osteoides Gewebe, das beinahe kalkfrei bleibt, gebildet wird. Sehr schön sind die Versuche Erdheims, in denen er zeigt, wie eng die Verknöcherung mit der Gegenwart der Nebenschilddrüsen verbunden ist. Werden an der Ratte die Nebenschilddrüsen entfernt und dann nach einer gewissen Zeit temporär implantiert und dann wieder entfernt, nachdem sie mit Erfolg eingeheilt waren, so zeigen die im Wachstum befindlichen Schneidezähne eigentümliche Zonen von nicht calcifiziertem Gewebe, das der Zeit entspricht, während deren die Nebenschilddrüsen ungenügend funktionierten, zunächst als sie noch nicht eingeheilt und noch nicht tätig waren, und nach der zweiten Exstirpation der Drüsen.

Nach solchen Experimenten ist es schwierig, die Annahme zu umgehen, daß die Nebenschilddrüsen etwas mit dem Kalkstoffwechsel zu tun haben. Toyofuku bestätigt und beschreibt ausführlich die Veränderung am Dentin, die Erdheim gefunden hat. Morel meint, daß die Parathyreoidektomie an erwachsenen Tieren die Heilung von Knochenbrüchen nicht hintanhält, wenn sie das auch an jungen Tieren in exquisiter Weise tue.

Aus all diesen ziemlich widersprechenden Befunden geht hervor, daß das letzte Wort über die Rolle des Calciums und der andern anorganischen Substanzen bei der Tetanie noch nicht gesprochen ist. Das meiste spricht doch für die Ansicht, daß die Störung letzten Endes bedingt ist, durch die Kalkverarmung der Gewebe, und die mannigfachen Fragmente bilden zusammen eine verlockende Theorie, der in der Tat keine unbestreitbaren Argumente gegenübergestellt werden können. Allerdings liegt auch manche Beobachtung vor, die diese Theorie nicht stützt; die Tatsachen mögen immerhin noch andere Deutungen zulassen. Selbst theoretisch ist es schwierig, die Resultate präzis zu skizzieren, die die Probe auf eine solche Theorie liefern und die Beweiskraft so geringfügiger Veränderungen in den Calciumverhältnissen ist fraglich infolge der komplexen Natur der Bedingungen, unter denen das Calcium normalerweise im Körper vorkommt.

Wenn wir in der Lage wären, die Aufnahme des Kalks durch den Verdauungskanal zu verfolgen und zu zeigen, daß die Gesamtausfuhr durch den Urin und Stuhl während längerer Beobachtung vermehrt wäre, so könnten wir schließen, daß das Calcium tatsächlich aus den Geweben stammt. Reichlich wird der Kalk der Nahrung durch die Faeces ausgeschieden, ohne jemals resorbiert worden zu sein. Da die

Ausscheidung notorisch unregelmäßig ist, so müssen lange Perioden untersucht werden. Wenn, um das zu vermeiden, überhaupt kein Futter gegeben wird, so wird die Ausscheidung des Kalks möglicherweise durch den Hungerzustand verändert, wiewohl wir offenbar unter solchen Bedingungen eine äußerst regelmäßige Calciumexkretion erhalten.

Im Skelett haben wir einen großen Vorrat von Kalk, der ohne Zweifel im Falle der Not verfügbar wird, so daß das Calcium der Exkrete aus dem unveränderten Nahrungskalk, dem Kalkvorrat in den Knochen und in anderen Geweben und dem resorbierten, im Blute kreisenden stammt.

Zu einer Steigerung der Ausscheidung gehört eine rasche Abführung nach Darm und Niere — oder eine Steigerung in den zirkulierenden Flüssigkeiten. Es ist verständlich, daß wenigstens eine Zeitlang, im Laufe einer gesteigerten Ausfuhr, das Blut weniger Kalk als normal im einen Falle, mehr als normal im anderen Falle enthält. Man kann nicht sagen, wie lange die Knochen und anderen Gewebe die Calciumausscheidung unterhalten können. Aber es scheint doch unvermeidlich, daß eine Zeit kommt, zu der Blut und Gewebe verarmt sein müssen, und nach der es dann schwerlich mehr möglich ist, daß die Ausscheidung die Norm übersteigt. Entsprechend also dem Stadium, in dem sich die Kalkauslaugung der Gewebe befindet, hätten wir vermehrte Ausscheidung, verbunden mit exzessivem oder subnormalem Kalkgehalt des Blutes. Dann ist es begreiflich, daß eine solche Erschöpfung eintritt, daß auch bei leichter Entfernung des Kalkes aus dem Blute seine Kalkkonzentration so niedrig wird, daß die Ausscheidung verringert ist. Das alles muß abhängen von zwei Faktoren: der Geschwindigkeit, mit der das Calcium aus den Geweben entfernt wird, und der Geschwindigkeit, mit der es in Darminhalt und Urin ausgeschieden wird.

Aber ein anderer Faktor muß bei dieser Hypothese noch berücksichtigt werden. Das Calcium wird nicht wirklich entfernt, sondern so gebunden, daß es für die Gewebe unbrauchbar geworden ist. Es ist begreiflich, daß es durch eine Bindung aus der Reihe der „aktiven" Calciumverbindungen ausscheiden und für die Gewebe nutzlos werden kann. Dann mögen unsere groben chemischen Methoden der Calciumdarstellung durch Veraschung usw. einen Überfluß an Calcium im Gehirn ergeben, während es tatsächlich nur in einer unlöslichen Form vorhanden ist, die seine normale vitale Rolle verändert hat. Man kann das alles ganz einfach veranschaulichen: wir stellen das den Geweben verfügbare Calcium durch eine gefärbte Lösung dar und haben es in 2 Flaschen, die Knochen und Gewebe darstellen sollen; aus diesen führen Röhren, die mit Stopfen und Hähnen versehen sind, zu einer gemeinsamen Röhre. Eine andere Flasche enthält klares Wasser und möge in diese Röhre führen, die keinen Stopfen mit Hahn besitzt. Ein anderer Hahn am Boden der Röhre stellt den Weg der Ausscheidung vor. Wird dieser letzte Hahn weit geöffnet, so wird klares Wasser ablaufen. Wenn einer der oberen Hähne offen ist, so wird

sich das Wasser färben. Je weiter der untere Hahn geöffnet ist, desto heller wird die Farbe des Ablaufs sein (entsprechend der Abnahme der Konzentration des Calciums im Blut mit dem Ansteigen der Ausscheidung). Ist der Hahn beinahe geschlossen, so wird der Ablauf stark gefärbt sein, solange in den oberen Gefäßen noch Farblösung enthalten ist. Nun soll dieser untere Hahn seitlich angebracht sein, die Röhre möge sich nach oben fortsetzen und zurückführen und die leicht gefärbte Flüssigkeit durch die Gefäße darüber durchwaschen: ein Teil der Farbe wird zurückkommen, der größere Teil aber ausgewaschen werden. Die Verhältnisse werden noch komplizierter, wenn die Farbe z. B. durch Kaliumpermanganat dargestellt wird und wenn zum klaren Wasser ein Zusatz von Oxalsäure kommt. Dann haben wir in Zirkulation etwas von der Farbe (aktives Calcium), und etwas von demselben Material, das wegen seiner neuen Verbindung farblos ist. Ob wir zu einer genauen Schätzung über den Inhalt eines der oberen Gefäße gelangen können aus der Bestimmung des gesamten gefärbten Materials, das durch den seitlichen Hahn zum Boden fließt, oder aber aus dem Gehalt der zirkulierenden Flüssigkeit, das ist schwer zu sagen. Dem Referenten erscheint es klüger, im gegegebenen Falle der Tetanie zu entscheiden, was die Oxalsäure unseres Modells vorstellt.

Wechselwirkung der Organe mit innerer Sekretion.

Es würde zu weit führen, die ganze Frage der funktionellen Beziehungen zwischen verschiedenen Organen mit innerer Sekretion zu diskutieren. Unsere Kenntnisse sind so spärlich hinsichtlich fast all dieser Organe, daß wir zu den unsichersten Schlüssen über ihre Funktion gezwungen sind. Es ist sehr möglich, daß Veränderungen, die wir auf ihre Tätigkeit oder das Fehlen solcher Tätigkeit beziehen, in Wirklichkeit von etwas anderem abhängen, oder daß der Mechanismus, mittels dessen sie aufeinander reagieren, sehr verschieden von dem ist, den wir voraussetzen. Der Ausdruck „Hormon" ist viel gebraucht worden und gemißbraucht in dem Bestreben, eben die Art und Weise zu bezeichnen, wie Veränderungen in der Tätigkeit eines Organs abhängen von der Tätigkeit anderer. Es ist so einfach, anzunehmen, daß ein Organ in seiner Funktion eine chemische Substanz produziert, die in die Blutbahn übergeht und die Tätigkeit eines anderen weckt, daß dieses Schlagwort dazu dient, den kompliziertesten Prozeß zu erklären. Tatsächlich kennen wir nur sehr wenig von dem wahren Wesen oder auch nur von der Existenz dieser Substanzen, und die Beweise, die für die Existenz der ersteren erbracht waren, sind in der neuen Arbeit von Frank erschüttert worden. Nichtsdestoweniger bleibt das Phänomen bestehen, die Vorstellung „Hormon" ist vielleicht so gut wie jede andere, vorausgesetzt, daß wir darin nur eine Arbeitshypothese erblicken. Etwa in diesem Sinne haben Falta, Eppinger und Rudinger, Caro, Balint und Molnar, Guleke und andere das merkwürdige Gleichgewicht zu erklären versucht, das im Körper offenbar herrscht, und

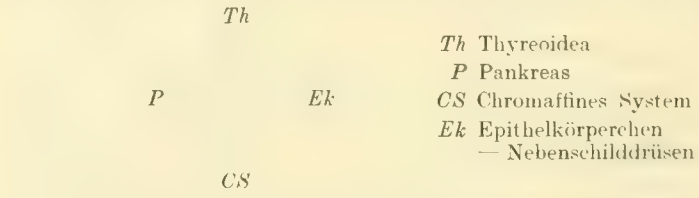
das Versagen der Funktion, das sich bei einer Störung dieses Gleichgewichts einstellt. Dieses ganze Gebiet hat seine Darsteller gefunden und ist so bekannt, daß nur der Teil, der sich auf das Fehlen der Nebenschilddrüsenfunktion bezieht, hier Erwähnung finden soll. In ihrer ersten Arbeit haben Eppinger, Falta und Rudinger eine Theorie aufgestellt, die auf zahlreichen Experimenten basiert, und auch weiterhin durch Experimente belegt. Diese Theorie gipfelt in dem Satze, daß zwischen der Schilddrüse und Pankreas und zwischen Pankreas und chromaffinem System ein intensiver Antagonismus herrscht, während Schilddrüse und chromaffines System gegenseitig ihre Tätigkeit fördern. Dies fanden sie so eng an die Innervation dieser Organe geknüpft, daß sie glaubten, sie könnten zwischen autonomem und sympathischem Nervensystem den nämlichen Antagonismus feststellen, soweit sie die Prozesse der inneren Sekretion zu kontrollieren hätten, etwa so, wie das der Fall ist bei den Drüsen mit Ausführungsgängen. Diese These stützte sich auf Experimente an diesen Drüsen, angestellt mit Hilfe von Mitteln, deren lähmende oder reizende Wirkung auf die Nervenendigungen bekannt war. Seit der Zeit sind auch die Nebenschilddrüsen mit hinein in den Kreis von Beziehungen gezogen worden. Jene Autoren hielten es für möglich, auch auf sie und ihre Funktion diese Vorstellung auszudehnen.

Caro findet, daß das Serum bei einem Tiere, das eine Schilddrüsenextrakteinspritzung erhält, einen Überschuß an Adrenalin aufweist, während ein Antagonismus zwischen Nebennieren und den Geschlechtsdrüsen besteht. Er stellt eine Formel auf mit Rücksicht auf alle diese Organe, die sie in zwei Gruppen scheidet: der ersten gehören die Organe an, die die Tätigkeit des sympathischen Systems beeinflussen, die Schilddrüse, das chromaffine System und die Hypophyse. In der zweiten Gruppe, den Antagonisten des sympathischen Systems, befinden sich Pankreas, Nebenschilddrüsen und Ovarien. Die erstere vermehrt den Hungereiweißzerfall, mobilisiert Kohlehydrate, erhöht den Fettstoffwechsel, Wasser- und Salzwechsel, und erhöht die galvanische Erregbarkeit der Nerven. Pankreas und Nebenschilddrüse haben in jeder dieser Beziehungen eine hemmende Wirkung; das gilt auch in manchen Fällen vom Ovarium. Balint und Molnar kommen zu ähnlichen Resultaten und finden, daß Schilddrüsenextrakt die mydriatische Wirkung des Adrenalins vermehrt, das Pankreas dagegen sie hemmt. Im übrigen betreffen ihre Beobachtungen die antagonistische Tätigkeit des Adrenalins und der Schilddrüse zur Strychninvergiftung, während Pankreasextrakt dieselbe verstärkt, und kommen zum Schluß, daß es sich hierbei um eine vasokonstriktorische Wirkung handelt.

Falta und Rudinger finden bei ihren Untersuchungen über Arbeitertetanie, daß das Adrenalin erheblich den Blutdruck steigert, und erklären das damit, daß die Nebenschilddrüsen gewöhnlich die sympathischen Einwirkungen hemmen, und daß bei ihrer Insuffizienz diese Hemmung fortfällt. In ihrem Referat auf dem 26. Kongreß für innere Medizin stellen sie fest, daß, während die Tetanie zum Teil in

der Übererregbarkeit des motorischen und sensiblen peripheren Neurons besteht, ebenso Störungen im Kohlehydratstoffwechsel eintreten. Bei Tieren ruft Adrenalin keine Glykosurie hervor, die Assimilationsgrenze ist nicht herabgesetzt. Bei der Tetanie dagegen ruft Adrenalin Glykosurie hervor, auch wenn die Schilddrüse mitentfernt ist, und es besteht alimentäre Glykosurie. Die Aufgabe der Nebenschilddrüse ist es gewöhnlich, das Pankreas zu unterstützen und die Wirkung des chromaffinen Systems zu hemmen, so daß deren Zerstörung die Kohlehydratmobilisierung steigert. Sie halten es auch für möglich, daß eine Verbindung zwischen der Übererregbarkeit der sympathischen und cerebrospinalen Nerven durch Vermittelung des chromaffinen Systems besteht, da diese Erregbarkeit durch Adrenalin gesteigert werden kann. An myxödematösen Hunden nimmt die galvanische Erregbarkeit deshalb ab, weil der Verlust der fördernden Eigenschaften der Schilddrüsen zu einer verminderten Tätigkeit des chromaffinen Systems führt, und diese zu einer relativen Überfunktion der Nebenschilddrüsen.

So wird es wahrscheinlich, daß normalerweise die Nebenschilddrüsen auf das sympathische System hemmend wirken, wie sie das für den Stoffwechsel tun, so daß nach ihrer Zerstörung ein Übererregungszustand in den peripheren motorischen und sensiblen Neuronen Platz greift, der sich in tetanischen Spasmen äußert. Falta und Kahn geben Versuche an zur Demonstration der gesteigerten Tätigkeit des Adrenalins bei der Tetanie und glauben, daß es sich dabei um die gesteigerte Erregbarkeit der Myoneuralbrücke sympathicotropen Reizen gegenüber handelt. Guleke auf der anderen Seite findet, daß Exstirpation der Nebennieren die Tetanie zum Stillstand bringt, die von der Exstirpation der Nebenschilddrüsen mit den Schilddrüsen herrührt. Werden die Nebenschilddrüsen allein entfernt, so bringt die Zerstörung der Nebennieren keine Änderung hervor. Er schließt daraus auf einen Antagonismus zwischen Nebenschilddrüse und Schilddrüse, gleich dem zwischen Nebenschilddrüse und chromaffinem System, in dem die erstere hemmend, die letztere erregend auf das sympathische System wirkt. Er erdenkt ein neues Schema, um diese Korrelation folgendermaßen darzustellen:



in dem nebeneinander die antagonistischen Organe, gegenüber die sich verstärkenden Organe liegen.

Falta und seine Mitarbeiter haben so eine höchst anziehende Arbeitshypothese aufgestellt, die den neuen Tatsachen besonders gut angepaßt ist, und die, wie sie sagen, neuen Untersuchungen den Weg zu zeigen vermag. Es ist unmöglich, eine der Erklärungen, die für diese Beobachtungen in all ihrer Kompliziertheit gegeben wurden, ab-

zuleugnen, trotzdem man nur sehr vage darüber orientiert ist, was Hemmung, was Förderung eigentlich bedeuten. Wenn der Verlust der Funktion der Schilddrüse die galvanische Erregbarkeit herabsetzt, so müssen wir dann schließen, daß die Ursache die ist, daß der Verlust der Schilddrüse die hypothetische Förderung des chromaffinen Systems beseitigt? Und wenn wir zugeben, daß das chromaffine System nur schwach wirksam ist, soll das besagen, daß weniger Adrenalin als sonst gebildet wird? Wenn ja, bedeutet das eine relative Überfunktion der Nebenschilddrüsen? Und bewiese das, daß normalerweise die Nebenschilddrüsen das sympathische System hemmen? Folgt daraus, daß sie normalerweise die Tätigkeit der motorischen Nerven hemmen? So viele dieser Annahmen erforderten einen befriedigenderen Beweis, als er allein durch die Beobachtung gegeben ist, daß nach der Zerstörung einer Drüse gewisse angenommene Funktionen einer anderen in Erscheinung treten. So z. B. die wechselseitige Förderung zwischen Schilddrüse und Nebennieren.

Nach dem Wegfall der Schilddrüse ist es schwierig, durch Einführung von Zucker, durch Äther oder durch Adrenalininjektionen eine Glykosurie zu erhalten, und deshalb nimmt man von der Schilddrüse an, daß durch sie die Funktion der Nebennieren gefördert bzw. durch ihren Wegfall vermindert wird, d. h. daß weniger Adrenalin produziert wird. Umgekehrt kann die Einspritzung von Schilddrüsenextrakt die Entstehung einer Glykosurie begünstigen, und deshalb glaubt man die Funktion der Nebenniere und damit die Produktion des Adrenalins angereizt zu haben. Aber stimmt das alles?

Die Glykosurie muß in solchem Falle abhängen von dem Grad der Hyperglykämie, die resultiert, wenn die Glykolyse beeinträchtigt ist. Es sollte möglich sein, das nachzuweisen mit Hilfe der Cohnheimschen Methode des Nachweises der Glykolyse durch Vermischen von Pankreasextrakt, Muskelsaft und Zucker. King findet, daß die Beimengung von Schilddrüsensaft die Glykolyse hemmt und den Zucker in Lösung erhält. Ist es deshalb nicht plausibler, diese wechselseitige Förderung zwischen Schilddrüse und chromaffinem System als viel besser erklärt zu betrachten durch die Vorstellung, daß das Adrenalin den Zucker mobilisiert; weiterhin, daß entsprechend der Menge von Schilddrüsenextrakt, die mit der Cohnheimschen Mischung von Zucker und Pankreassaft im Muskel zusammentrifft, mehr oder weniger Glykolyse eintritt? Mit anderen Worten, die innere Sekretion des Pankreas wirkt wie ein Katalysator; die Schilddrüsensekretion interferiert mehr oder weniger mit ihr, wie die Blausäure mit anderen Katalasen tut.

Diese Idee wurde durch Mac Callum mit Rücksicht auf die innere Sekretion des Pankreas folgendermaßen ausgedrückt: es scheint wahrscheinlicher, daß das chromaffine System nichts mit der Tätigkeit des Pankreas, sondern etwas mit der Mobilisierung des Zuckers zu tun hat; daß ebenso das Pankreas nicht mit der Hemmung der Wirkung der Nebennieren beschäftigt ist, sondern damit, die Glykolyse des freien Zuckers zu bewerkstelligen, während die Schilddrüsensekretion, wenn sie im Übermaß stattfindet, die glykolytische Tätigkeit des Pankreasferments stört.

Keiner dieser Einwände soll das Gewicht der Faltaschen Befunde herabsetzen den Tatsachen gegenüber, die eine Erklärung erheischen, sondern bloß eine einfachere Erklärung vorschlagen: die Sekretionen dieser verschiedenen Drüsen beeinflussen sich gegenseitig chemisch in der Mischung, in der sie ihre Wirkungen offenbaren, eher als daß sie auf anderem Wege die funktionierende Tätigkeit der andern Organe beeinflussen. Demgegenüber ermuntert der isolierte Befund Caros, daß das Serum von Tieren, die mit Schilddrüsenextrakt behandelt sind, einen Überschuß an Adrenalin zeigt, zu aussichtsreichen Versuchen in Verbindung mit allen anderen Organen.

Pathologische Anatomie der Tetanie.

Pathologisch-anatomische Untersuchungen bei der Tetanie haben so dürftige und unsichere Befunde ergeben, daß sie gut in Verbindung mit den einzelnen Krankheitstypen gebracht werden können.

Bei der experimentellen Tetanie nach der Parathyreoidektomie wurden einige Veränderungen in den Organen beschrieben. Diejenigen am Zentralnervensystem sind unbedeutend oder wenigstens kaum mit unseren histologischen Methoden dargestellt worden. Gozzi fand an Hunden nach Parathyreoidektomie regelmäßig schwere Veränderungen in der Leber, die im Verhältnis zu der Schwere und Häufigkeit der tetanischen Symptome standen und Capillaren sowohl als Leberzellen betrafen. Alquier und Theveny beobachten ebenso Läsionen in Leber und Niere wie auch Capobianco und Mazziotti, die feststellen, daß die allgemeinsten Veränderungen in den Nieren Hämorrhagien in die Glomeruli sind, Anhäufung von Lymphocyten und Leukocyten zwischen den Tubuli mit hyalinen und granulierten Zylindern in ihren Lumina. Das Epithel der Tubuli ist wie bei Vergiftungen degeneriert. Massaglia beobachtete Schädigungen der Niere, die Albuminurie machten, und Nazari fand in Fällen von gastrischer Tetanie eine seltsame Verkalkung der Tubuli contorti. Die meisten Beobachter fanden keine besonderen Alterationen in den Organen bei der experimentellen oder spontanen Tetanie mit Ausnahme der Fälle von infantiler Tetanie.

Ausbreitung der Tetanie.

Sehr wenig ist seit der Monographie Rudingers 1909 hinzugekommen zu unserer Kenntnis der allgemeinen Pathologie der Tetanie. Einige Arbeiten, die die Entwicklung der Tetanie an mehreren Familiengliedern betreffen, müssen doch berichtet werden. Ascenzi berichtete von einer Familie mit 5 Kindern, von denen 2 ausgeprägte Tetanie zeigten, 1 Epilepsie, 1 Laryngospasmus, und alle eine Verzögerung in der Entwicklung. Coler kannte 2 Schwestern, beide schmal und schwach, von denen eine an Rachitis, die andere an den verschiedensten fieberhaften Krankheiten litt. Beide boten alle Zeichen und Symptome der Tetanie mit schweren Anfällen von spastischen

Krämpfen. Calcium lacticum blieb ohne Effekt. Schiffer gibt einen ausführlichen Bericht über eine Familie mit 5 Kindern, von gesunden Eltern stammend, die alle tetanisch waren. Gelegentlich der Autopsie wurden bei einem von ihnen 4 Nebenschilddrüsen gefunden ohne jegliches Zeichen von Hämorrhagie. Suiz fand in Triest häufig familiäres Vorkommen der Tetanie und beschreibt 3 Familien; in 2 von diesen wurden Mutter und Töchter, in der dritten Bruder und Schwester von Tetanie befallen. Er nimmt hereditäre Schwäche der Nebenschilddrüsen an, da eine anatomische Grundlage für jede Insuffizienz fehlte. Man muß die akquirierte von der hereditären Insuffizienz trennen. In dieser Beziehung ist der Hinweis von Fuchs u. a. auf einen etwaigen Zusammenhang zwischen Ergotinvergiftung und Tetanie interessant.

Tetanie während der Schwangerschaft bzw. Lactation: Viele Fälle von Tetanie sind im Laufe der Schwangerschaft oder während der Lactation kürzlich von Dakin, Schmiedlechner, Adler und Thaler, Groß, Gottschalk, Guiziotti, Hodlmoser, Neumann, Meinert, Nathan, Pick, Thomas, Zanfroginini u. a. berichtet worden. Aber Neues wurde durch diese Beobachtungen nicht erbracht. Die Originalexperimente von Vassale sind die in jeder Beziehung klarsten. Er fand, daß die Tetanie an partiell parathyreoidektomierten Tieren während der Schwangerschaft oder nach der Entbindung in die Erscheinung tritt, wenn mehrere Junge geworfen wurden, so daß die Lactation stark war. Mit der Unterbrechung der Lactation schwand die Tetanie. Eine befriedigende Erklärung besitzen wir nicht, wenn es sich nicht als richtig herausstellen sollte, daß die wesentlichste Erscheinung sowohl bei der Schwangerschaft als der Lactation der Verlust an Kalksalzen ist.

Infantile Tetanie: Weit mehr Aufmerksamkeit war der infantilen Tetanie gewidmet worden, und die letzten Jahre haben weiterhin neues Tatsachenmaterial und neue Hypothesen beigebracht, die sich in der Hauptsache einerseits mit dem Wesen der Krankheit und im besonderen dem Calciumgehalt der Nahrung beschäftigen, andererseits mit gewissen Läsionen der Nebenschilddrüsen, die sich hauptsächlich an Kindern finden. Der ersten dieser Fragen wurde Erwähnung getan bei der Diskussion über die Rolle des Calciums. Sein Einfluß ist bei Kindern nicht so klar wie in den Experimenten, in denen es intravenös appliziert werden kann, da die Resorption im Darmkanal der Kinder noch immerhin unsicher ist. Z. B. stellte Schabad fest, daß bei der Ernährung mit reiner Kuhmilch eine Kalkverarmung stattfinden muß, da überhaupt nur 30 Proz. resorbiert werden, im Gegensatz zu 70 Proz. von der menschlichen Milch. Die Frage ist allem Augenschein nach komplexer, als es bei oberflächlicher Betrachtung scheint, und kann nicht durch eine einfache Untersuchung über den Kalkgehalt der Nahrung beantwortet werden.

Mit einer Anerkennung dieser Tatsache haben manche Untersucher nach einer anatomischen Grundlage für die Erklärung der Spasmophilie gesucht, und wie wohl bekannt, gelang es Erdheim die Gegenwart von Hämatomen in den Nebenschilddrüsen darzutun, die er auf As-

phyxie oder Trauma während der Geburt bezog und die eine Störung der Drüsenfunktion darstellen. Die Arbeit Yanases, der diese Ansicht kräftig unterstützte, hat einen Platz in vielen Referaten gefunden. Nach jener Zeit hat der Befund solcher Hämorrhagien in den Nebenschilddrüsen von Kindern, die keine Zeichen von Tetanie aufwiesen, und ihr Fehlen bei anderen mit ausgesprochenen Symptomen manche Autoren veranlaßt, ihre Wichtigkeit in Frage zu stellen. Thiemich bestritt ihr Vorkommen in seinen Fällen von Tetanie, dagegen stellt Strada unter Bezugnahme auf die Arbeiten von Erdheim, Schmorl, Pepere, Verebeley, Getzowa u. a., die sie ebenso beschrieben, fest, daß in seinen 4 Fällen von infantiler Tetanie Hämorrhagien zu finden waren, mit starker Beeinträchtigung des Parathyreoidgewebes. Haberfeld fand an Kindern Zeichen von Hämorrhagien in den Nebenschilddrüsen, die manchmal lange Zeit, ja bis 12 Monate, unverändert liegen geblieben waren oder Narben und Pigmentierung hinterlassen hatten. Ihr Effekt ist nicht allein das Zugrundegehen des Gewebes, sondern auch eine Wachstumsbeschränkung der Drüsen. Vielleicht handelt es sich nur um einen prädisponierenden Faktor für die Tetanie, während die unmittelbare Ursache Infektion oder Intoxikation ist. Er macht auf die wichtige Tatsache aufmerksam, daß das Fehlen von Klappen in den Venen der Nebenschilddrüsen die Entstehung dieser Hämorrhagien begünstigt. Auf der anderen Seite fand Auerbach Hämorrhagien in den Nebenschilddrüsen von 76 Proz. aller, inklusive der tetanischen Kinder. In seiner zweiten Arbeit gibt er uns eine ausführliche Zusammenstellung:

13 Kinder mit normaler Nervenerregbarkeit;				davon 8	mit Hämorrhagien
				5	ohne „
5	„	„ anodaler Nervenübererregbarkeit;	„	4	mit „
				1	ohne „
5	„	„ kathodaler „	„	5	mit „
				0	ohne „
10	„	„ klinisch manifester Tetanie;	„	8	mit „
				2	ohne „

Er findet Pigment und Cysten als Residuen von Hämorrhagien mit häufig sekundären Hämorrhagien in und um diese Cysten. Aus diesen Befunden schließt er, daß Asphyxie bei der Geburt das Vorkommen von Hämorrhagien nur in 76 Proz. dieser Fälle erklärt, und daß man nicht sicher sein kann, daß die Tetanie der Kinder gleichbedeutend mit der bei Erwachsenen, oder daß die Hämorrhagien der Nebenschilddrüsen ihre Ursache sind.

Bliß fand keine Hämorrhagien in 2 Fällen von kindlicher Tetanie, aber 3mal Hämorrhagien unter 13 gesunden Kindern, und stellte eine Beziehung zwischen solchen Veränderungen und der Tetanie in Abrede. Großer und Betke sind derselben Ansicht und finden Hämorrhagien der Nebenschilddrüsen nicht nur nach Geburtsasphyxie, sondern im späteren Leben nach entzündlichen Prozessen und Autointoxikationen, und ziehen den Schluß, daß diese nichts zu tun haben mit der in-

fantilen Tetanie, die ihrerseits wieder etwas anderes ist als die Tetanie nach Parathyreoidektomie. Oppenheimer fand bei einem Fall von infantiler Tetanie mit elektrischer Übererregbarkeit in keiner der 4 Nebenschilddrüsen eine Blutung, wohl aber gewisse Spalträume, die er als rupturierte Lymphgefäße deutete. Königstein fand normale Nebenschilddrüsen bei einem 9monatigen Kind mit Tetanie, dagegen Blutungen in den Nebenschilddrüsen bei einem Kind, das an Enteritis ohne Tetanie starb. Eckert fand Tetanie bei Kindern mit normalen Nebenschilddrüsen. Jörgensen findet es bemerkenswert, daß, wenn die Tetanie etwas mit Blutungen in die Nebenschilddrüsen zu tun habe, sie sich erst 3 bis 4 Monate nach der Hämorrhagie einstelle. Er führt alle Argumente gegen die Bedeutung der Blutungen bei der Tetanie an und bespricht schließlich einen Fall von ausgesprochener Tetanie bei einem 7monatigen Kinde, bei dessen Nebenschilddrüsen Serienschnitte vollkommen normales Verhalten ergeben hatten.

Im ganzen ist der Beweis für das ursächliche Verhältnis dieser Blutungen zu der Tetanie nicht sehr überzeugend, besonders wenn man bedenkt, wie wenig Parathyreoidgewebe am Tier dazu gehört, um es normal zu erhalten. Daß solche hämorrhagische Zerstörungen sich als prädisponierende Faktoren fühlbar machen können, kann niemand leugnen; aber es scheint doch, daß andere Prozesse wie Autointoxikationen die direkte Ursache der Tetanie sein müssen.

Das wird noch wahrscheinlicher, wenn wir unsere Aufmerksamkeit anderen Formen der Tetanie zuwenden, die im späteren Leben auftreten und bei denen adäquate Veränderungen in den Nebenschilddrüsen niemals gefunden werden.

Tetanie bei Intoxikationen: Gewisse giftige Substanzen rufen Symptome hervor, die ähnlich, wenn nicht identisch sind mit denen der Tetanie, und in manchen Fällen befällt ein tetanischer Anfall eine Person, von der man weiß, daß sie für solche Symptome prädisponiert ist. So beschreibt Chvostek einen tetanischen Anfall als Resultat einer Tuberkulininjektion. Skene beobachtete Tetanie bei einem idiotischen Kind, das mit Schilddrüsenextrakt behandelt worden war, und das unglücklicherweise 25 grain (= 1,25 g) auf einmal verschluckt hatte. Mehrere Stunden später entwickelte sich die Tetanie und wiederholte sich, so oft die große Dosis verabreicht wurde. Der Verfasser ist der Ansicht, daß das Thyreoidin die Ausfuhr an Kalk steigert und in diesem Fall den Kalkvorrat erschöpfte. (!) Haenel fand nach leichten Vergiftungen tetanieähnliche Symptome, und mehrere Autoren beschrieben solche nach Phosphorvergiftungen (Routa, Stransky, Wirth). In diesen Fällen handelt es sich um Magendilatationen oder sonst prädisponierende Ursachen, und Wirth ist der Ansicht, daß der Phosphor die Nebenschilddrüsen schädige. In seinem Falle fand er erhöhte elektrische und mechanische Erregbarkeit und Acetonurie.

Rudinger ist der Ansicht, daß die Tetanie durch Gifte verursacht werden kann bei gleichzeitig bestehender Parathyreoidinsuffizienz, und erwähnt in dieser Beziehung speziell Ergotin, Kalomel usw. Pick hatte

Ergotin als ätiologisches Moment bei der Tetanie der Schwangeren im Verdacht und spricht sich gegen die Verwendung des Ergotins ebenso wie gegen die von Chloroform in der geburtshilflichen Praxis aus. Neuerdings hat Fuchs ernstlich das Ergotin als die Ursache der in Wien so verbreiteten idiopathischen oder Arbeitertetanie beschuldigt. Er findet, daß Ergotismus, Spasmen, Parästhesien, Katarakt, Polyurie, fibrilläre Zuckungen und trophische Störungen der Haare, Nägel und Haut mit mechanischer Muskelüberempfindlichkeit erzeuge. Er erklärt das Vorkommen der Tetanie im Winter auf Grund der Tatsache, daß der Roggen, der nach Weihnachten benutzt wird, reichlich Ergotin enthalte, das Ergotin im März abgestorben sei. Biedl diskutiert diese Arbeit und macht auf gewisse Diskrepanz im Bilde des Ergotismus und der Tetanie aufmerksam. Allerdings war er imstande, elektrische Erregbarkeit durch subcutane Ergotininjektion hervorzurufen.

Es ist bekannt, daß Asphyxie oder partielle Anämie leicht die Nervenerregbarkeit verändert, so daß die Formel sich der der Tetanie nähert mit gesteigerter Erregbarkeit gegenüber dem Kathoden- und Anodenöffnungsschlag. Da die meisten dieser Gifte die Blutzufuhr zur Peripherie oder die Oxydationsprozesse stören, so scheint es möglich, daß der Symptomenkomplex, den sie hervorrufen, davon abhängen muß, eben wie es denkbar ist, daß die Symptome der Tetanie selbst die Wirkung einer Oxydationsstörung darstellen können.

Gastrische Tetanie: Der Stand unserer Kenntnis von dem Wesen der gastrischen Tetanie ist ziemlich spärlich. Es wurde darüber wenig in den letzten Jahren publiziert, und die Publikationen, die erschienen sind, bestanden im wesentlichen aus Berichten von Fällen, gewöhnlich mit Gastroenterostomie und Heilung der Symptome. Jeder der Verfasser hat die Literatur referiert und die drei Haupttheorien über die Ätiologie diskutiert, von denen keine eine befriedigende Erklärung gibt. Solche Referate finden sich in Arbeiten von Amato, Albu, Bing, Cunningham, Dickson, Durand, A. Ferrannini, L. Ferrannini, Fenwick, Gatzky, Gumprecht, Rudinger und Jonas, Sievers u. a. Die Theorie einer Autointoxikation infolge Speiseretention im Magen hat manche Anhänger gefunden, und entschiedene Anstrengungen wurden gemacht, um das erwartete Gift im Mageninhalt oder im Urin zu finden. Durch komplizierte Methoden fanden einige Beobachter giftiges Material im Magen, andere fanden keines; manchmal handelte es sich um Hyperchlorhydrie, manchmal um ein Salzsäuredefizit. Nach manchen hängt die Produktion oder aber auch die Wirksamkeit eines Giftes von der sauren Reaktion ab. Andere finden basische Substanzen, Diamine oder Toxalbumine, denen sie die Symptome zuschreiben. Auch Aceton und verwandte Substanzen wurden im Urin gefunden.

Bouveret und Devic finden, daß das Gift nichts mit Bakterien und nichts mit Hyperacidität zu tun hat, und daß es wahrscheinlich ist, daß im Laufe der Peptonisierung ein Ptomain entsteht, alkohollöslich, ja daß vielleicht der Alkohol mit seiner Bildung zusammenhänge.

Unter genau den nämlichen Bedingungen findet Gumprecht kein Toxin, und obwohl der Urin toxischer ist als in der Norm, ist er der Ansicht, daß kein besonderes Tetaniegift erkennbar ist. Da sind die einen und die anderen, die sich so oder so für die aktive Beteiligung eines Magengiftes entscheiden. Aber ihre Befunde sind vage und zweifelhaft.

Die Arbeit von Kaufmann hat in dieser Beziehung zu geringe Beachtung gefunden. Er spricht aus, daß die Gastroenterostomie unmittelbare Hilfe bringt und hält diese Wirkung für unmöglich, wenn die Wirkung der Operation darin bestände, giftige Substanzen im Mageninhalt nach dem Intestinum gelangen zu lassen. Er erinnert an die von F. Müller gefundene Tatsache, daß keine Tetanie entsteht, wenn stagnierendes Material resorbiert wird, dagegen wohl, wenn es durch Erbrechen oder Auspumpen entfernt wird. Es ist tatsächlich richtig, daß in manchen Fällen Auswaschen die Symptome hintanhält, aber in anderen, z. B. in dem Falle Colliers, folgt die Tetanie direkt der Auswaschung oder exzessivem Erbrechen. Aus diesem Grund, meint Kaufmann, sei es der reichliche Verlust von Chloriden, der die Tetanie verursache.

Ohne Kenntnis dieser Arbeit führte Mac Callum 1908 bis 1909 Serien von Experimenten an Hunden aus, bei denen der Pylorus vollständig verschlossen, der Hund durch eine Duodenalfistel ernährt und der Magen gründlich ausgewaschen wurde. Jeden Tag waren große Mengen von Magensekret, reich an Chloriden, angehäuft, was zweifellos zu einem Verluste des Körpers an Salzen führte. Die Tiere zeigten in einigen Fällen ausgesprochene tetanische Symptome. Dr. Poole demonstrierte an neueren Versuchen der nämlichen Art eine abnorm hohe elektrische Erregbarkeit der Hunde. Der Gedanke war damals der, daß der Verlust an Chloriden in solcher Menge das aktive Calcium, das dem Körper zur Verfügung steht, beeinträchtigt. Die Arbeit ist sehr unvollständig und mag vielleicht nach der verkehrten Richtung ausgegangen sein; aber sie stimmt überein mit den guten Resultaten der Gastroenterostomie.

Guerra Coppioli hält den Verlust an Salzsäure für das ätiologische Moment der Tetanie, indem es die Alkalinität des Blutes vermehrt.

Jonnesco und Großmann, Jurgensen, Kuckein und Fleiner beziehen sich auf die Idee Kußmauls, daß der Wasserverlust infolge Erbrechens und der daher verminderten Absorption von den Därmen die wahre Ursache der Tetanie sei, da in manchen Fällen die Symptome durch rectale Wassereinläufe behoben werden können. Richartz sah weniger guten Erfolg von diesen Einläufen und meint, daß die Befunde nicht für diese Eindickungstheorie sprächen. Wenn es sich um ein gastrisches Toxin handelte, so müßte es allmählich resorbiert werden. Es könnte sein, daß es nie in großer Menge im Magen vorkommt. Da die jedesmalige Einführung einer Magensonde einen tetanischen Anfall auslöste, so erwägt er die reflektorische Entstehung der Spasmen.

Die Frage bleibt in der Schwebe und macht noch reichliche Studien notwendig. Die Nebenschilddrüsen fanden sich normal oder aber ver-

größert, wenn sie zur Untersuchung kamen (Erdheim, Kinnicutt, Mac Callum). Mac Callum sprach die Ansicht aus, daß die Hypertrophie wahrscheinlich von einer vermehrten Sekretion herrühre, die durch die Ansprüche bedingt ist, die die Tetanie an diese Drüsen stellt.

Intestinale Tetanie: In einer Anzahl von Fällen ist die Tetanie vergesellschaftet, mehr mit intestinalen als gastrischen Störungen, und diese erfordern wieder eine andere Erklärung, obwohl eine der Erklärungen, die man für die gastrische Form gegeben hat, auch hier in Betracht kommt. Fleiner sah einen Fall von Achylia gastrica, bei dem die Nahrung leicht den Magen passierte; Fett wurde bis zur Verseifung gespalten, aber nicht resorbiert. Die Stühle waren sehr reichlich, und die Diurese war vermehrt. Er bezog diese Fälle auf die Kußmaulsche Theorie von der Bluteindickung. Ewald und Jacobsohn sahen ebenso einen Fall von Steatorrhoe mit tetanischen Anfällen, die jedesmal auftraten, wenn der Stuhl fest wurde. Sie waren befriedigt von dem Befunde einer ptomainartigen Substanz im Urin. Langmead beschrieb 3 Fälle von Dilatation des Colon sigmoideum mit Retention und Zersetzung der Faeces. Eins dieser Kinder zeigte die charakteristischen Symptome von intestinalem Infantilismus, und alle zeigten Tetanie, die verschwand, sobald der Darm ausgewaschen wurde. Schließlich berichtet Quosig über einen Fall analog dem von Fleiner, mit Achylie und Diarrhöe, den er aber einer Autointoxikation zuschreibt.

Osteomalacie und Rachitis.

Wir haben gezeigt, daß die Nebenschilddrüsen einen entscheidenden Einfluß auf den Ossifikationsprozeß haben, der nur unvollständig und ohne Verkalkung vor sich geht, wenn diese Drüsen fehlen, ganz wie es der Fall ist bei Rachitis und Osteomalacie (Erdheim, Morel, Toyofuku). Es liegt deshalb nahe, diese Krankheiten, die durch Störungen in der Verkalkung und Verknöcherung charakterisiert sind, zu Alterationen der Nebenschilddrüsen in Beziehung zu bringen. Das ist tatsächlich von Erdheim bewiesen worden, der in 6 oder 7 Fällen von ausgesprochener Osteomalacie die Nebenschilddrüsen hochgradig vergrößert, und wenn nicht vergrößert, so doch von Gewebsmassen durchsetzt fand, die den Eindruck von Neubildungen machten. Er sah das als Ausdruck einer Mehrfunktion an, die durch die Bildung irgendeiner Substanz, z. B. im Ovarium, erforderlich wurde, zu deren Neutralisation die Nebenschilddrüsen nötig wurden.

Der Zusammenhang dieser Fälle mit Tetanie ist schon lange richtig erkannt worden, und Erdheim berichtet Fälle von Blazicek, Freund, Weber, Schultze, Hecker, Panse, Hoennicke. Ein anderer Fall ist von Schlesinger beschrieben worden, bei dem die Tetanie mit der Osteomalacie 7 Monate nach dem Wochenbett verschwand. Schönborn versuchte die Behandlung in einem solchen Falle mit Nebenschilddrüsenextrakt, aber ohne Erfolg. Strada fand eine von 4 Nebenschilddrüsen bei Osteomalacie vergrößert. Auch Bauer fand Knötchen

eines jungen Nebenschilddrüsengewebes in jeder der 4 Drüsen mittels der Altmannschen Methode, und vermutet das Vorkommen von Osteomalacie in jenen Fällen von Nebenschilddrüsentumoren, die schon beschrieben wurden. Schmorl fand nur in einem Fall von Osteomalacie eine Hyperplasie der Nebenschilddrüse. Marek wandte in seinem Falle von Osteomalacie, in dem er während der 4. Gravidität Kaiserschnitt und Kastration ausführte, Adrenalininjektion an, von dem Gedanken geleitet, daß es sich hier um die Insuffizienz des chromaffinen Systems handelte. Jede Injektion war von Tetanie begleitet. Seine Erklärung ist sehr unbefriedigend; denn wenn man annimmt, daß es sich dabei um eine Insuffizienz des chromaffinen Systems handelt, so sollte man glauben, daß dabei eine relative Überwertigkeit der Nebenschilddrüsen vorhanden ist. Denn wir haben es mit dem umgekehrten Fall zu tun von normaler Schwangerschaft, bei dem Neu und Hofbauer eine Zunahme im Adrenalingehalt des Blutes mit relativer Nebenschilddrüseninsuffizienz fanden. Plausibler scheint der Gedanke, daß bei der Osteomalacie eine Substanz gebildet wird, die alle Kräfte der Nebenschilddrüsen zu seiner Neutralisation verbraucht. Bei diesem Verhältnis einer relativen Insuffizienz kann die Einführung eines Überschusses von Adrenalin einen tetanischen Anfall auslösen in der Weise, wie Falta es beschreibt; obwohl in den zahlreichen Fällen, in denen nach Bossis Angaben bei der Osteomalacie Adrenalin gegeben wurde, offenbar keine entschiedenen Symptome von Tetanie beobachtet wurden. Hier scheinen die Alterationen der Nebenschilddrüsen und die Tetanie ganz nebensächlich im Vergleich mit dem ursächlichen Faktor, der die Osteomalacie und die Calciumverarmung bedingt. Wahrscheinlich könnte dasselbe von der Rachitis gesagt werden, die so gewöhnlich mit Tetanie vergesellschaftet vorkommt (Schüller).

Trophische Störungen in anderen Geweben.

Veränderungen an den Nägeln, der Haut und der Linse sind in mehreren Tetaniefällen beschrieben worden und bilden wohl Analoga zu denjenigen an den Knochen und Zähnen. In der Tat beobachtete Spieler bei einem 10jährigen Kind, bei dem die Tetanie mit 8 Jahren eingesetzt hatte und jedes Frühjahr mit Anfällen von Laryngospasmus wiederkehrte, solche Veränderungen. Pineles macht auf die Mannigfaltigkeit dieser Phänomene bei Kindern aufmerksam im Gegensatz zu Erwachsenen, und kann Fälle von Hoffmann und Peters anführen. Seitdem hat Fischl einen Fall beschrieben mit Atrophie und allmählichem Verlust der Daumennägel. In Spielers Fall handelte es sich um eine Hypoplasie des Schmelzes und Dentins derjenigen Zähne, die sich während der Tetanie entwickelten, während die vorher gebildeten vollkommen normal waren.

Nathan sah einen kurzen Anfall von Tetanie während der Schwangerschaft einer jungen Frau, bei der Parästhesien in den Fingerspitzen einhergingen mit Blasen und Geschwürsbildungen und Vernar-

bungen. Gleichzeitig war ein beginnender Katarakt vorhanden. In dem Falle Meinerts entwickelte sich ebenso die Tetanie in der Schwangerschaft und führte zum Verlust der Finger- und Zehennägel. Die vierte und fünfte Schwangerschaft verlief normal, aber in der sechsten trat Tetanie während des 4. Monats auf, die Nägel, die regeneriert waren, fielen in Stücken ab und ein bilateraler Katarakt entwickelte sich.

Zirm wirft die Frage auf, ob der Tetaniekatarakt (Schichtstar), der auch bei Kindern vorkommt, eine Wirkung des tetanisch veränderten Ciliarmuskels auf die Zirkulation, eine trophische Störung anderer Art oder eine direkte Giftwirkung ist, wie beim Naphthalinstar. Er neigt zu der letzteren Ansicht, da auch Ergotinvergiftung Katarakt verursacht, eine Tatsache, die im Licht der neuen Fuchsschen Arbeit über das Ergotin als ätiologischen Faktor bei der Tetanie von Interesse ist. Osterroht veröffentlicht einen Fall von bilateralem Katarakt bei einer Frau, bei der Tetanie mit der ersten von 8 Geburten 19 Jahre zuvor begonnen hatte. In diesem Falle war der Katarakt mit Pannus und Conjunctivitis gepaart, wobei auch Atrophie der Schilddrüse bestand. Vogts Fall betraf eine Frau, die, seit einer Strumektomie 20 Jahre vorher, an Krämpfen gelitten hatte. Ein doppelseitiger Katarakt entwickelte sich ohne Nägel- und Hautveränderung. Auch Vogt vergleicht ihn mit dem Ergotinkatarakt und nimmt Bezug auf mehrere ältere Arbeiten über denselben Gegenstand (Lantzberg, Schüller, Wagenmann, Westphal).

Natürlich brauchen diese Störungen einige Zeit zu ihrer Entwicklung, was Pineles für den wahrscheinlichen Grund ihrer seltenen Vorkommen bei der infantilen Tetanie ansieht. Deshalb können sich natürlich auch im gewöhnlichen Tierexperiment ähnliche Veränderungen nicht einstellen: nur in Erdheims Experimenten mit lang unterhaltener Tetanie bei Ratten fand man den Beginn eines Katarakts.

Eine plausible Erklärung für ihre Pathogenese gibt es nicht.

Therapie der Tetanie.

Hinsichtlich der Therapie sind nur wenig befriedigende Resultate erzielt worden. Lusena berichtet 1898 über gute Resultate an Hunden nach Implantation von Nebenschilddrüsen oder der Injektion von Nebenschilddrüsenextrakt. Verfütterung von Nebenschilddrüsengewebe hatte offenbar wenig Einfluß, und glänzende Resultate sind nicht erzielt worden. Cristiani führt erfolgreiche Transplantation bei Ratten an. Einige Jahre später kam man therapeutisch etwas weiter. Mac Callum konnte 1903 zeigen, daß die intravenöse Injektion von Nebenschilddrüsensaft an Hunden für eine Zeitlang die Symptome der Tetanie beseitigte. Tastende Versuche wurden und werden noch mit der Verfütterung von Schilddrüsenextrakt gemacht, manchmal mit gutem Resultate, was man sich damit erklärte, daß Nebenschilddrüsengewebe in den Schilddrüsen enthalten war, aus denen der Extrakt hergestellt wurde. Freilich sind nach Pineles solche Dosen ohne Bedeutung.

Vassale hat ein sogenanntes „Parathyreoidin" hergestellt, womit er und andere bemerkenswerte Resultate erzielte. Bircher, Löwenthal und Wiebrecht und Berkeley haben die Symptome durch Verfütterung bestimmter Nebenschilddrüsenextrakte beseitigt, während Rensburg und Rey, Escherich und Pineles davon recht unbefriedigt sind. Berkeleys Methode besteht darin, die Nucleoproteide zu verfüttern, die aus dem wässerigen Extrakt von Rinder-Nebenschilddrüsen ausfallen, und hält sie für das wirksame Prinzip. In einigen Fällen leichter Tetanie hatte er gute Resultate.

Netter, Quest u. a. gaben Calciumsalze bei der Tetanie mit guten Resultaten trotz der Theorie Stoeltzners, daß die infantile Tetanie zu guter Letzt durch Calciumsalze verursacht sei. Mac Callum und Voegtlin fanden, wie berichtet, daß intravenöse oder reichliche orale Verabreichung von Calcium die Symptome der experimentellen, durch Parathyreoidektomie erzeugten Tetanie prompt beeinflussen. Mitunter ist es möglich gewesen, ein Tier für lange Zeit am Leben zu erhalten. Eine definitive Heilung findet natürlich niemals statt, wenngleich die Symptome maskiert werden. Seine Anwendung muß also hauptsächlich auf die Überwindung der Symptome bei dringendem Notfalle oder, in protrahierten Fällen, sich auf die Linderung der Symptome beschränken. Es gibt Fälle, in denen sich die Tetanie an eine Strumektomie anschließt. Die Nebenschilddrüsen sind dabei nicht sämtlich zerstört, wohl aber so geschädigt worden, daß eine Tetanie ausgelöst wurde. Wenn die Heftigkeit der Symptome durch die Anwendung des Calciums überstanden wird, so könnten vielleicht in einer gewissen Zeit die Nebenschilddrüsen ihre Funktion wieder erfüllen. Der Arzt ist aber noch recht hilflos vor einem chronischen Fall von Tetanie, wo keine entschiedene Indikation für das ärztliche Eingreifen in Betracht kommt. Bei gastrischer Tetanie, wo die Gastroenterostomie oft so prompte Hilfe bringt, und in der Schwangerschaftstetanie, wo die Beendigung der Schwangerschaft oft die Tetanie beseitigt, ist die Aussicht hoffnungsvoller. Calcium kann unbegrenzt gegeben werden, aber in einer beträchtlichen Zahl von klinischen Berichten wird es als völlig wirkungslos hingestellt.

Transplantation von Nebenschilddrüsen.

Seit der Arbeit von Halsted 1907 sind mehrere Vorschläge zur Transplantation von menschlichen und tierischen Nebenschilddrüsen gemacht worden. Halsted beschreibt die Einpflanzung der Nebenschilddrüsen in die Milz und Schilddrüse bei Hunden. Im selben Jahre berichteten Pfeiffer und Mayer über eine erfolgreiche Transplantation der Nebenschilddrüsen eines Hundes in die Rectumscheide, die den Ausbruch der Tetanie bei dem Tiere verhütete. Cimoroni benutzte das Omentum und fand, daß die überpflanzten Nebenschilddrüsen das Leben ziemlich lange unterhalten können, daß sie aber bald resorbiert waren, wonach das Tier kachektisch wurde und starb. Böse heilte postoperative Tetanie durch Implantantion menschlicher Nebenschild-

drüsen unter die Bauchhaut. Hermann und Harvey erprobten das viscerale Peritoneum als eine geeignetere Transplantationsstätte als die Milz und stellten fest, daß Isotransplantation möglich sei.

Halsted veröffentlichte 1909 in einer zweiten, äußerst sorgfältigen Arbeit, daß Autotransplantation, d. h. die Verpflanzung der Nebenschilddrüsen an ein und demselben Tiere möglich ist, aber nur, wenn man eine Nebenschilddrüseninsuffizienz erzeugt und ein absolutes Bedürfnis nach dem Organ vorlag. Selbst wo das transplantierte Organ samt den übrig bleibenden annähernd die zur Aufrechterhaltung der Gesundheit genügende Masse darstellte, wollte es nicht Wurzel fassen, so daß bei den erfolgreichsten Hundeversuchen eine Insuffizienz und Neigung zur Tetanie zurückblieb. Isotransplantationen gelangen ihm nicht; er stellt die Resultate Biedls in Frage, dessen Implantationen mit fremden Nebenschilddrüsen gut gelangen, ohne daß freilich ihre Funktionstüchtigkeit geprüft wurde. In Halsteds Versuchen zeigte eine mikroskopische Untersuchung, wie gering die überpflanzten Gewebsmassen waren, deren Entfernung nach Monaten, während deren das Leben allein durch sie erhalten worden war, prompt die verhängnisvolle Tetanie hervorrief.

Danielsen und Landois beschreiben diese Operation und referieren die deutsche Literatur mit dem Ende, daß eine Transplantation möglich ist. Leichner und Kohler führen homoioplastische Transplantationen aus und finden, daß das transplantierte Gewebe ebenso wie das Schilddrüsengewebe langsam resorbiert wird, man deshalb nur von temporärem Erfolg sprechen kann. Immerhin gewinnen einige übrig gebliebenen Reste Zeit zur kompensatorischen Hypertrophie. Krabbel referiert den ganzen Gegenstand. Er überpflanzte die menschliche Drüse in einem Fall von idiopathischer Tetanie mit gutem Resultat; der Patient starb aber bald danach an Tuberkulose, was die Vermutung nahelegt, daß eine Tuberkulose der Nebenschilddrüsen die ursprüngliche Ursache der Tetanie war.

Phleps berichtete über solche Transplantation bei der Tetanie in der Schwangerschaft und bei anderen Zuständen, die zwar nicht zu einer Tetanie geführt hatten, wohl aber zu trophischen Störungen, wie Katarakt usw. Er erwähnt 2 Fälle, in denen die Implantation gute Resultate gab. Saar gibt eine Beschreibung der operativen Technik und hält das präperitoneale Gewebe für den besten Platz. Eiselsberg, Garré und Poole haben diese Drüsen ebenso verpflanzt mit mehr oder weniger gutem Erfolg. Obwohl das Prinzip an sich gut begründet sein mag, so sind doch manche Einzelheiten auszuarbeiten; dann dürfte eine solche Therapie sich allmählich aussichtsreicher gestalten.

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Modifications of the Abel vividiffusion apparatus.

By W. G. MacCallum, M.D. and R. A. Lambert, M.D.

[*Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*]

The apparatus for the study of the circulating blood by a process of diffusion devised by Drs. Abel, Rowntree and Turner has impressed us as such a brilliant stroke of inventive genius that we have hastened to imitate it and apply it. The scope of this method is perhaps not yet fully appreciated, but it has interested us particularly in affording a hopeful way of studying the inorganic constituents of the blood, especially in connection with tetany where we think that calcium plays a considerable rôle. The results of these experiments are as yet entirely incomplete but we wish to describe several modifications in the technique which may have been tried and discarded by Dr. Abel but which seem to us to be helpful.

The branched glass end pieces which allow the current of blood a choice of several paths are extremely difficult to make and are very fragile. Unless they are made very precisely, one path becomes easier than another in which blood may circulate slowly or stagnate. With this arrangement blood passes once only through the length of the apparatus and back again, but since the stream bed, with these many possible channels, is very wide, the current must be relatively slow.

We have constructed two or three machines in which the blood circulates back and forth eight or ten times before returning to the vein. In the first model the connections were made as in a steam radiator with "U" tubes of glass set in discs of hard rubber fixed on a central glass rod. All this was enclosed in a water jacket. In spite of our fear that the blood pressure might be insufficient to force the blood through this long course (about 80

inches) it works well, and we have carried on an experiment for five hours. The difficulty in preventing leaks where the celloidin tube is tied on the glass we have obviated by first wrapping the joint with rubber adhesive plaster and then tying. The difficulty in tying on the tubes of the inner row when the "U" tubes are ranged round a disc, we have removed by spreading out the "U" tubes flat in a square frame made of rubber and glass which can be turned over when it is necessary to tie the lower row. When the sheaf of tubes is enclosed in a water cylinder plugged at both ends, it is impossible to remedy a leak except by stopping the experiment, but we have simply laid our square frame in an enamel pan of the fluid covered with a glass lid and can reach any part of it at any time without disturbing the circulation.

Hirudin is very expensive and we have therefore defibrinated the blood of our animals realizing the possible objections to this. Instead of starting with the celloidin tubes full of salt solution they are filled with defibrinated blood of another normal dog and enough of this is kept in a funnel or tank connected with the inlet tube to allow some blood from the machine to run through the outlet tube into the vein while a similar amount is bled from the carotid and defibrinated. This is poured into the funnel and the process repeated till the blood no longer clots when the inlet tube is connected with the carotid canula and circulation proceeds. So rapid is the torrent of blood through such a continuous channel that we have twice connected the inlet and outlet tubes directly with artery and vein without defibrinating and kept up the circulation for an hour without the formation of any clot. If the blood pressure sinks, through faulty etherization or other reason the blood clots, as happened in another experiment.

The most important and difficult part of the problem is the calculation of the proper fluid in which to immerse the apparatus. In our effort to abstract calcium we have tried several and are at present using a fluid calculated to correspond as closely as possible with Abderhalden's published analysis of the inorganic constituents of the dog's blood with the omission of the calcium.

With this apparatus dialysis, at least in so far as the inorganic substances are concerned, is rapid and satisfactory.

This paper was read at the Seventeenth International Congress of Medicine, London, August 1913.

[Friday Morning, August 8]

Chairman—Professor J. Lorrain Smith, M.D., F.R.S.

SECTION III

GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY

INDEPENDENT PAPER

ON CERTAIN RELATIONS BETWEEN LIPOID SUBSTANCES AND THE ADRENALS

By Dr. HUGH A. STEWART,[1] New York

The function of the adrenal gland and especially of the adrenal cortex has for so long been a subject of dispute that any evidence bearing upon it must be welcome. In the course in pathological physiology given to the students in 1912 an attempt was made to demonstrate the character of the rapidly fatal effects of extirpation of both adrenal glands. Rats were used in this experiment, although it is well known that they often survive the operation because they so often possess accessory glands. Of these experiments one which resulted in the longest survival of the animal proved to have been the case of a pregnant rat. This was at any rate sufficient to suggest the possibility that pregnancy might have some influence upon the course of the symptoms, and similar operations were performed on other animals, chiefly cats and rabbits with a few guinea-pigs. The results may be presented in tabular form for brevity, but it may be said that in most instances the pregnant and lactating animals survived the extirpation of the adrenal glands much longer than normal, non-pregnant, or male animals. It would perhaps be less confusing to eliminate from the table all animals which died of infection, anæsthesia, or from such accidental causes, because in these the reason for death was so obvious that they detracted hardly at all from the clear impression that if the adrenal extirpation were performed completely and without

[1] Dr. Stewart died of scarlet fever on March 29 after a brief illness, leaving behind him no connected account of the work in which he was engaged. The essential observations had been made some months before his death, but he hesitated to publish any statement concerning them until he could gather confirmatory evidence of their correctness through several methods of attack. Only a small part of the experiments which he had planned had been entered upon when he died, and practically nothing was completed to his satisfaction. Nevertheless, although he was by no means ready to publish his results, it seems best to me to do so now as well as I can from the notes which he left. His opinions and impressions are in a great part lost, but it will be possible to indicate in a general way the trend of his thought with regard to the facts observed. It is hoped that we may be able to work out to some conclusion later the several problems which he had encountered.—W. G. MacCallum.

TABLE I

Pregnant and Lactating Animals—First Series

No.	*Animal.*	*Condition.*	*First adrenal removed.*	*Second adrenal removed.*	*Days of duration of life after second operation.*	*Results.*
			1912	1912		
1	Cat	Lactating	May 13	May 20	10 days	Kittens 3 weeks old. Lively until day of death. Lost weight.
2	Cat	Lactating	May 13	May 20	11 days	Weak, emaciated. Cold, twitching.
3	Rabbit	Lactating	May 24	May 28	6 days	Paralysed at end. Cold and weak.
4	Rabbit	Lactating	May 28		2 days	Infarct of kidney.
5	Rabbit	Lactating	May 29	June 3		Both ovaries removed. Still alive Oct. 12, 1912.
6	Guinea-pig	Lactating	June 1		1 day	Died of infection.
7	Cat	Pregnant	June 4		7 days	Killed.
8	Guinea-pig	Lactating	June 6	June 10	Died on table	
9	Guinea-pig	Lactating	June 7	June 11	2 days	No infection.
10	Guinea-pig	Pregnant	June 8	June 12	7 days	Wound infection.
11	Rabbit	Lactating	June 12	June 18	1 day	Wound infection.
12	Cat	Pregnant. Removed ovaries, June 12			Died June 22	Infection.
13	Rabbit	Lactating	June 14	June 18	1 year	Alive Sept 30. Died June 1913.
14	Cat	Lactating	June 15	June 24	9 days	Infected.
15	Guinea-pig	Lactating	June 15		5 days	Infected.
16	Cat	Pregnant. Removed ovaries, June 17	June 22	June 26	1 day	Infected (?).
17	Guinea-pig	Lactating	June 18	June 22	2 days	No infection.
18	Cat	Lactating	June 18	June 22	6 days	Infection.
19	Guinea-pig	Lactating	June 20	June 24		Still alive, Sept. 30.
20	Cat	Pregnant. Removed ovaries, June 21	June 24	June 28	7 days	No infection.
21	Cat	Pregnant	June 25	June 27	Died on table	
22	Rabbit	Delivered young dead	June 26	June 29	2 days	No infection.
23	Cat	Pregnant	Sept. 13	Sept. 18	10 days	Infection.

TABLE II

Pregnant and Lactating Animals—Second Series

No.	*Animal.*	*Condition.*	*First adrenal removed.*	*Second adrenal removed.*	*Days of duration of life after second operation.*	*Results.*
			1913	1913		
1	Cat	Pregnant. 3 kittens Jan. 13	Jan. 14	Jan. 18	9 days	No infection. No adrenal remains
2	Cat		Feb. 5	Feb. 10	1 day	No infection.
3	Cat	Pregnant	Feb. 7			Killed.
4	Cat	Pregnant	Feb. 7	Feb. 11	12 hours	Infection.
5	Cat	Pregnant	Feb. 9	Feb. 13	3 days	Infection.
6	Cat	Pregnant	Feb. 11	Feb. 13	2 days	Infection.
7	Cat	Pregnant	Feb. 11	Feb. 15	Killed on table	
8	Cat	Pregnant	Feb. 12	Feb. 17	2 days	No infection
9	Cat					Died under ether.
10	Cat	Premature kittens	Feb. 18	Feb. 22	4 days	No infection.
11	Cat	Lactating. 4 kittens	Feb. 20	Feb. 24	2 days	Killed by lanolin injection.
12	Cat	Lactating	Feb. 24	Mar. 7	7 days	Infection.
13	Cat	Lactating	Mar. 10			Killed.
14	Cat	Lactating	Mar. 12	Mar. 17	2 days	Distemper.
15	Cat	Lactating	Mar. 13	Mar. 18	2 days	Sponge in abdominal cavity.
16	Cat					Killed
17	Cat	Lactating	Mar. 24			
18	Cat					Killed.

Average duration of life after second operation, 4·4 days.

TABLE III

Control Animals

No.	Animal	Condition	First adrenal removed.	Second adrenal removed.	Days of duration of life after second operation	Results.
			1912	1912		
1	Cat	Male	May 23	May 27	1 day	
2	Cat	Male	May 26			Killed.
3	Cat	Male	May 27	June 1	1 day	Peritonitis from first operation
4	Cat		May 29	June 3	5 days	
5	Cat	Female	May 29	June 3	5 days	
6	Guinea pig	Female	June 1	June 5	3 days	
7	Cat	Male	June 1	June 5	3 days	Rapid loss of weight.
8	Cat	Male	June 4	June 8	2 days	
9	Cat		June 6	June 10	4 days	
10	Rabbit		June 7	June 11	1 day	
11	Cat	Removed pancreas				Killed.
12	Cat	Female Pancreatectomy, June 15	June 18	June 20	1 day	
13	Rabbit	Female	June 20	June 24	Died	
14	Cat		June 20	June 24	2½ days	
16	Cat	Female (?)	Oct. 9	Oct. 14	1 day	
17	Cat	Female	Oct. 9	Oct. 14	1 day	
18	Cat	Male	Oct 9	Oct 14	2 days	Killed.
19						
20	Cat		Oct. 17	Oct. 21	Died same night	
21	Cat	Male	Oct 18	Oct. 22	1 day	
22	Cat	Female	Oct 18	Oct. 22	1 day	
23	Cat		Oct 14	Oct. 22	3 days	Lipoids.
25	Cat	Male	Oct 28	Nov. 7	3 days	Lipoids.
26	Cat	Male	Oct 23	Nov. 7	2 days	Lipoids.
27	Puppy					Died.
28	Rabbit		Oct. 31	Nov. 7	1 day	Lipoids.
29	Cat	Male	Nov. 11	Nov. 14		No lipoids.
30	Cat	Male	Nov. 11	Nov. 14	1 day	Cadmium chloride.
31	Cat	Male	Nov. 11	Nov. 14	4 days	Perfused heart. Lipoids.
32	Cat	Male	Nov. 15	Nov. 21	3 days	No lipoids.
33	Cat		Nov. 15	Nov. 21	3 days	No lipoids.
34	Cat		Oct. 23	Nov. 16 (transplant)	1 day	Lipoids.

35	Cat		Nov. 19	Nov. 25		Died at second operation. Perfusion.
36	Cat		Nov. 19	Nov. 23	2 days	
37	Cat	Male	Nov. 21	Nov. 23	2 days	Used for salt perfusion.
38	Cat	Female				
39	Rabbit		Nov. 27 1913	Dec. 1 1913	6 days	No lipoids.
40	Cat	Female	Jan. 9	Jan. 13	2 days	Injected blood, egg-yolk lecithin.
41	Cat	Male	Jan. 9	Jan. 13	3 days	Killed.
42	Cat	Male	Jan. 16	Jan. 21	1 day	
43	Cat	Female	Jan. 16	Jan. 21	2 days	Cholesterin analysis of blood. No lipoids.
44	Cat	Female	Jan. 21			Died on table.
45	Cat	Female	Jan. 27	Jan. 31	1 day	
46	Cat	Female				
47	Cat	Female	Feb. 3	Feb. 7	1 day	Bled for P_2O_5 estimation.
48	Cat	Female	Feb. 3	Feb. 7	1 day	Bled for P_2O_5 estimation.
49	Cat	Male	Feb. 4	Feb. 6	3 days	Killed.
50	Cat	Male	Feb. 10	Feb. 20	7 days	Bled for cholesterin determination.
51	Cat	Female	Feb. 25	Mar. 1	15 days	Injections of lanolin.
52	Cat	Female	Feb. 25	Mar. 1	2 days	Bled for analysis.
53	Cat	Female	Mar. 7			Injection of lanolin. Infected.
54	Cat	Male	Mar. 3	Mar. 7	4 days	Lanolin. Pneumonia.
55	Cat	Male	Mar. 5	Mar. 8	3 days	Lanolin. Pneumonia (?).
56	Cat	Male	Mar. 4	Mar. 8	5 days	Lanolin.
57	Cat	Male	Mar. 5		Killed	Weight of right adrenal, 240; weight of left adrenal, 330 2 days later.
58	Cat	Male	Mar. 8			Killed.
59						Pneumonia.
60						Ophthalmia; killed.

Average duration, exclusive of lanolin animals, 2·25 days

infection, the animal would live quite comfortably and without untoward symptoms for a much longer time than the control. Even when all cases are considered the average duration of life in the pregnant and lactating animals is 4·4 days as contrasted with 2·25 days, which is the average for the controls.

If now we accept only those cases both in controls and in pregnant and lactating animals which were not confused by infections and accidents, we should have the following table for cats:

TABLE IV

First Series

		Lived after operation. Days.	*Loss of weight.*
Cat 1	Lactating	10	
Cat 2	Lactating	11	300
Cat 18	Lactating	6 (infected	300
Cat 20	Pregnant	7	500
Cat 23	Pregnant	10	

Second Series.

Cat 1	Pregnant	9
Cat 8	Pregnant	2
Cat 10	Pregnant	4
Cat 12	Lactating	7 (infection)

Average 7·2 days

Controls

		Lived after operation. Days.			*Lived after operation. Days.*
Cat 1	Normal	1	Cat 22	Normal	1
Cat 4	Normal	5	Cat 23	Normal	3
Cat 7	Normal	3	Cat 25	Normal	3
Cat 8	Normal	2	Cat 26	Normal	2
Cat 9	Normal	4	Cat 30	Normal	1
Cat 12	Normal	1	Cat 32	Normal	3
Cat 14	Normal	2·5	Cat 33	Normal	3
Cat 16	Normal	1	Cat 43	Normal	2
Cat 17	Normal	1			
Cat 21	Normal	1			Average 2·2 days.

It is not only in the duration of life, however, that the differences become apparent, for the apathy, lowering of temperature, weakness, and phenomenally rapid loss of weight are all much delayed and mitigated in the pregnant or lactating animals as compared with the controls. Nevertheless, these symptoms do appear gradually and the loss of weight may, since the animal lives longer, be greater than in the case of the controls. The temperature usually falls several degrees, and in those animals which lived longest it reached a very low point (as in Cat 2 of Table I, where it stood at 28° C. during the day before death). The animal is unwilling to move and lies huddled with its head sunken so that its

nose touches the floor. It will not jump off a table and if pushed off falls awkwardly with its legs doubled under it. Occasionally distinct twitchings of the muscles were observed, but the electrical excitability of the nerves was not ascertained.

In several experiments to determine the effects of loss of the adrenals upon the heart, the hearts of normal and adrenalectomized animals were perfused side by side under conditions otherwise exactly identical. No difference in their behaviour could be observed; they beat at practically the same rate and for the same length of time.

Under such circumstances there seemed to be several possible factors which must be considered in attempting to explain the protective influence of pregnancy or lactation. It was thought (1) that the existence of the corpora lutea might compensate for the loss of the adrenals, since there is some vague resemblance between them, or (2) that the functional activity of the mammary glands might in some way act as a compensatory factor. Finally (3) there are various chemical changes in the circulating fluids and in the tissues of the body in pregnancy, notably an accumulation of lipoid substances, which might make such an animal less susceptible to the loss of the adrenals. None of these hypotheses has been worked out carefully. In only two cats, Nos. 16 and 20 of Table I, were the ovaries removed in addition to the adrenals. In one of these, 16, which was pregnant, and had kittens before the adrenals were destroyed, death resulted the day after the removal of the second adrenal. In the other (No. 20) the cat seems to have lived seven days after the removal of the second adrenal. Such experiments are too incomplete to allow of any conclusion on this point.

No experiments designed to eliminate the possible influence of lactation were carried out. A good deal of attention was paid, however, to the distribution of lipoid substances in the organs of these adrenalectomized animals, and it became quite evident that in many of them there occurred in the liver and sometimes in the kidney pale yellowish blotches which, on examination with the polarizing microscope, proved to be due to the presence of doubly refractive globules, which disappeared on heating and returned on cooling. These masses were not always endowed with the same brilliant double refractivity, but sometimes showed the Maltese cross only vaguely. In other cases, however, the appearance came out with the utmost clearness. Strangely enough, it was only in the control animals that this was studied. In cats 23, 25, 26, 28, 31, 34 the doubly refractive granules were found, while they were missed in Nos. 32 and 33 On account of their physical characters they were thought to be cholesterin esters. Efforts were made to apply micro-chemical reactions by the methods of Ciaccio, Fischler, Smith and others to determine more exactly the character of these substances, but they led to no satisfactory results at the time when they were interrupted. It was also attempted to isolate the different lipoid substances by the aid of solvents applied to frozen sections followed by staining methods, but although this seemed promising

it was not completed. The recorded results may be seen in the following table :

TABLE V

Animal.	*Organ.*	*Smith.*	*Ciaccio.*	*Neutral red.*	*Fischler.*	*Polarization.*
36	Liver	+		+		
37	Liver	+		+		
32	Liver	+				
33	Kidney					
33	Liver	+				
39	Liver	+		−	−	−
39	Kidney	+		−		
43	Liver	+		−		
43	Kidney	+		−		
Normal cat	Liver	+		−		
Normal rabbit	Liver	+		−		−
Normal rabbit	Liver		+	−	−	−

The impression was gained that reliance could be placed only in actual chemical estimations of the amounts of each fat present in the organ, since the staining methods seem so uncertain and overlap to such an extent. Cholesterin determined by the Windaus digitonin method gave as a result in the blood of a normal cat 0·064 % of free cholesterin. The blood of two cats (Table III, 50 and 52) from which the adrenals had been removed gave 0·0364 % of free cholesterin (0·076 % of combined cholesterin). Several possibilities of error, connected with the extraction, saponification, &c., must be considered in further studies of this sort. At least the result seemed to show a poverty of cholesterin esters in the circulating blood of the adrenalectomized animals.

On this account, and since it is known that cholesterin esters appear in increased quantity in the circulating blood of pregnant and lactating animals, it seemed possible that the protective influence of pregnancy in cases of adrenal destruction might be imitated by the artificial introduction of cholesterin esters. Defibrinated blood, egg-yolk, &c., were injected without result and cats were fed on egg-yolk without appreciable effect. Lanolin proves to be composed essentially of a mixture of cholesterin esters, and this was injected subcutaneously in Cat 51, beginning after the removal of the second adrenal. The animal received 3·5 grm. of lanolin subcutaneously every day. The effect was quite wonderful, for no sign of weakness appeared, and although it did not eat much it remained to all appearances well for fifteen days. The temperature was about 37 or 38° C. throughout, and after five or six days its appetite returned and it ate ravenously. The contrast between this cat, which ran to meet one at the door, and the others, which lay apathetic and cold in a corner, was very striking. On the fifteenth day the temperature fell to 36·8° and the cat died in the night. At autopsy there was no infection, but the oil was found in large amount in the inter-muscular tissues. No remnants of adrenal nor accessories were to be found. Cat 54, treated in the same

way, remained alive for four days after the second operation, but developed pneumonia and was killed. Cat 55 lived three days and died of pneumonia. Cat 56 lived five days with some symptoms of weakness the day before death occurred. No note of an autopsy was made.

While quite incomplete, this interrupted series is given because it seems most important in pointing out in a direct way the probable relation between the function of the adrenal and the presence of cholesterin esters in the blood. The conclusions to be drawn from these observations are not obvious, however. It cannot be stated that the adrenals form cholesterin esters which are deficient in their absence. It might be that the protective influence of the cholesterin esters in neutralizing some poisonous material (as shown in the case of chloroform or saponin poisoning) is necessary in the blood, and that the adrenal cortex stores up these lipoids for that reason. At any rate in the presence of abundant cholesterin esters in the blood the loss of the adrenal cortex is felt more slowly. The observations are therefore presented without any definite conclusion in the hope that the further study of these phenomena may give a more certain basis for such a conclusion.

Most of the earlier experiments were carried out with the aid of Dr. S. C. Harvey, whose assistance was greatly appreciated.

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SOAPS AS FERMENT-INHIBITING AGENTS.

STUDIES ON FERMENT ACTION. X.*

BY JAMES W. JOBLING, M.D., AND WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The use of the antitryptic index of the blood as a clinical test, particularly in cancer, and the pathological conditions in which ferment action appears to be held in abeyance, especially in caseation in tuberculosis and syphilis, in anemic infarcts, and in the exudate in lobar pneumonia, make the study of ferment-inhibiting substances of great importance. Many substances are considered to have the property of inhibiting the activity of enzymes, but in this paper we shall refer chiefly to the proteolytic enzymes acting in an alkaline medium.

Trypsin and leucoprotease lose their activity when heated at temperatures above 60° C., though according to Salkowski (1) trypsin when dry can be heated to 160° C. without being destroyed. Strong acids and alkalies quickly destroy trypsin, but Chittenden and Cummins (2) found it much more resistant to these agents in the presence of protein. Kudo (3) states that all the mineral acids are active in this respect, sulphuric acid being active in a dilution of one in a thousand, while the organic acids are much less so. Weiss (4) reports that while 0.05 per cent. sodium chloride increases the activity of trypsin, 10 per cent. decreases it. Most of the writers who have investigated the subject find that chloroform, toluol, and thymol inhibit the action of trypsin to a slight degree, but the necessity of a preservative in their experiments caused most workers to ignore this slight inhibition. Bayliss (5) and Abderhalden and Gigon (6) found that some of the products of tryptic activity possess the property of inhibiting the action of the ferment, the free amino acids being more active than the polypeptids. It has been known for several years that blood serum is capable of inhibiting the proteolytic power of trypsin, but in spite of the work of Camus and Gley (7), Charrin and Levaditi (8), Landsteiner (9), and others, we are still ignorant concerning its nature and significance.

In the course of our studies on ferment action we observed that soaps of certain fatty acids were capable of inhibiting the action of trypsin and leucoprotease. This observation seemed to be of importance, as soaps of a similar nature are present in the body, and the possibility of their acting in the same manner

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caused us to make a more careful study of the general phenomena relating to the inhibition of ferment activity by these substances.

Neumann (10), while studying the influence of a fat-rich diet on the digestion of infants, observed that sodium oleate inhibited the action of trypsin. For most of his work he used the Fuld-Gross technique. In some of our preliminary experiments we used the same technique, but soon discarded it in favor of the one which will be described later. According to the Fuld-Gross method the mixture of ferment and substrate is incubated for two hours and then made slightly acid by adding a few drops of a solution containing 5 per cent. acetic acid in 50 per cent. alcohol. The amount of digestion is determined by the degree of cloudiness, the more complete the digestion the clearer the contents of the tube after acidifying. Subsequent experience convinced us of the unreliability of this method, particularly when dealing with lipoidal substances. The acidifying not only coagulates the undigested protein, but also causes the lipoidal substances, particularly the fatty acids, to separate as flocculi, a condition which makes it impossible to determine the extent of proteolysis. Neumann added an excess of alcohol to cause a solution of the precipitated acids and thus solved one difficulty; but he added another by diluting the protein to such a degree that it became a problem to determine the extent of proteolysis.

Two ferments, trypsin and leucoprotease, were used in our work. The trypsin was obtained by extracting commercial pancreatin with N/50 sodium carbonate. After extraction for twenty-four hours, the solution was filtered, and the trypsin in the filtrate precipitated by the addition of nine volumes of alcohol to which sufficient acetic acid had been added to make the whole mixture slightly acid to litmus. This extraction and precipitation was repeated three times. The final product was much stronger than the original and the amount needed to cause complete digestion in the control tubes did not contain sufficient nitrogen to give a definite color when Nesslerized. The leucoprotease was prepared from human pus in the same manner, but the amount required to cause complete digestion in the control tubes contained more nitrogen than the concentrated trypsin. In each experiment the amount of nitrogen was determined in the control tubes and proper correction made in calculating the amount of total incoagulable nitrogen.

Edestin was used as a substrate for several experiments, but later it was discontinued in favor of a 1 per cent. casein solution, the latter being much more soluble in an alkaline medium, and in addition it was noted in precipitating the coagulable protein that a slight excess of acid caused the edestin precipitate to redissolve. After being incubated for the necessary time, the contents of the tubes

were acidified with a mixture containing 10 per cent. glacial acetic acid and 20 per cent. sodium chloride, and the tubes placed in boiling water for five to ten minutes. The mixtures were then filtered through kaolin and the incoagulable nitrogen was determined by the method recommended by Folin (11). The amount of nitrogen found in the control tubes was subtracted from each of the others and the results were given in percentages of total digestion.

The time allowed for incubation was not the same in all experiments. In our preliminary work we found that the concentrated trypsin, prepared as described above, deteriorated rapidly when once dissolved. For this reason the trypsin solution was prepared immediately before being used, one cubic centimeter representing 0.002 of a gram of trypsin. Several tubes containing only casein and trypsin were placed in the incubator at the same time as the tubes or flasks containing the mixtures of soaps. One of the tubes containing casein and trypsin was removed after one hour and tested. After that the other tubes were removed at intervals of fifteen minutes, also tested, and as soon as digestion was found to be complete the remainder were immediately removed, acidified, and boiled.

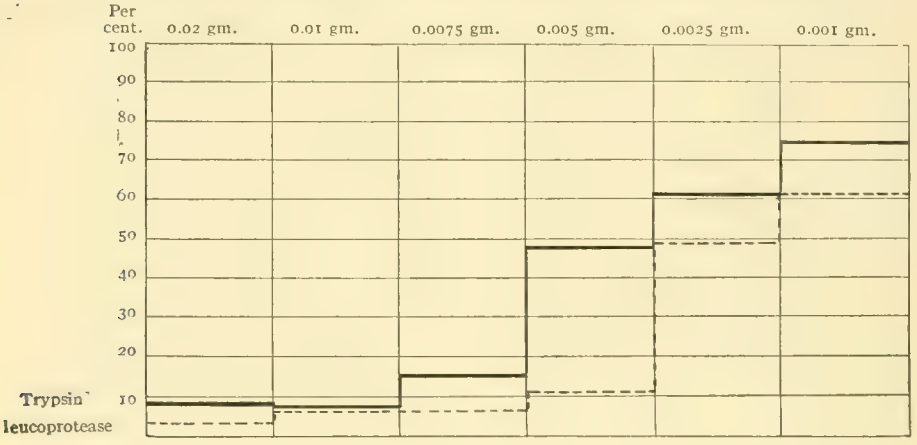
The protocols are not given for each experiment as the charts present the results more clearly. Table I gives the number of controls used in each experiment, and also the amount of nitrogen obtained by complete digestion and as a result of the inhibiting action of the soaps.

In most instances we did not know the identity of the acids used in the preparation of the soaps, but the isolation and identification of these with further work on their ferment-inhibiting action is now under way. The three supposedly pure soaps were Kahlbaum's preparations, and consisted of sodium oleate, sodium palmitate, and sodium stearate. On examination we found that the sodium oleate had become saturated, making it useless as an oleate, though it was used in several experiments as a representative of the saturated fatty acids.

The majority of the soaps were obtained by saponifying linseed, olive, cod-liver, hempseed, and castor oils, as these oils are known to contain a large number of unsaturated fatty acids. The oils were saponified with alcoholic potash, and the soaps extracted re-

peatedly with petroleum ether. The acids were then liberated by hydrochloric acid, taken up in ether, the ether evaporated, and the acids again saponified. This process was repeated and the acids were preserved pure until needed. The soaps used in the work were rarely more than two or three days old.

Later when it was found that the unsaturated fatty acids were the active inhibiting agents, the lead soap-ether method of obtaining these in higher concentrations was used. The separation of the saturated and unsaturated fatty acids by this method is not complete, but is sufficiently so to enable us to show marked difference between the action of the ether-soluble and insoluble fractions. In

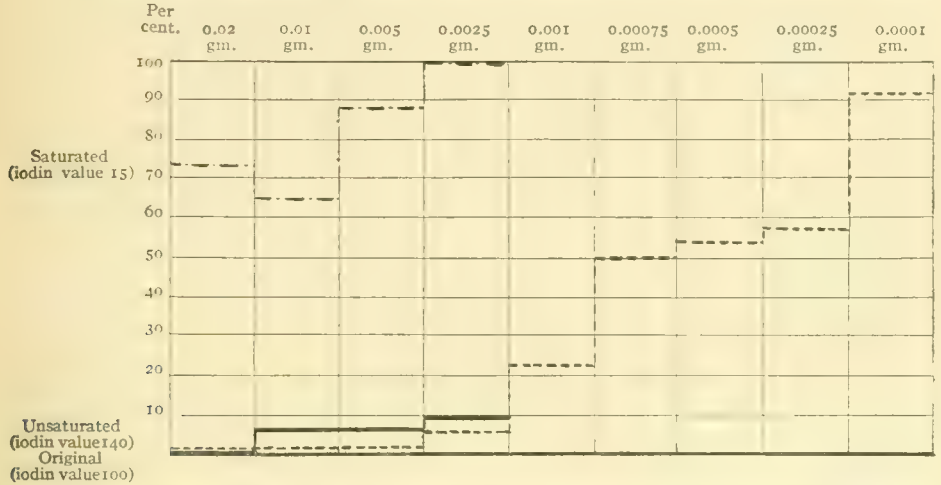


TEXT-FIG. 1. Effect of sodium oleate on tryptic and leucoproteolytic digestion.

nearly every instance the iodin value of the preparations was determined before they were used.

One of the first soaps investigated was that prepared from olive oil. Its influence on leucoprotease and trypsin is shown in text-figure 1. In this experiment the soap and ferment were mixed, incubated for thirty minutes at 37° C., and the casein was then added. This procedure was adopted in all the subsequent experiments. Text-figure 1 shows that even 0.001 of a gram of the soap is sufficient to affect materially the activity of both ferments. In this and in subsequent charts the control tubes showing 100 per cent. of digestion are not given.

Efforts were then made to determine if the inhibiting action displayed by these soaps was due to the saturated or unsaturated fatty acids present. Soaps from nearly all the oils were tested in a similar manner, but as uniform results were obtained, we shall give only one example. Text-figure 2 shows the results obtained with



TEXT-FIG. 2. Effect of saturated and unsaturated linseed soaps on tryptic digestion.

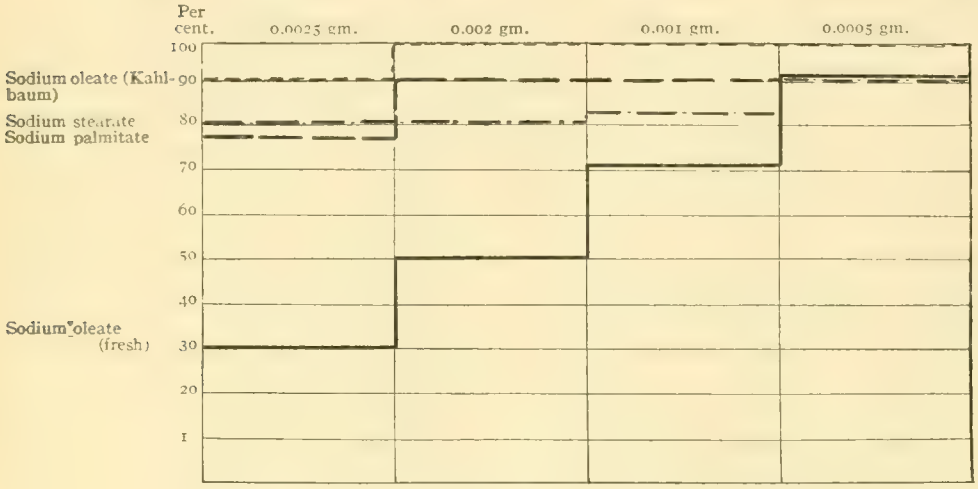
the soap prepared from linseed oil. The ether-lead soap method was used in separating the groups of acids. The chart indicates that the unsaturated fatty acids are the active inhibiting agents. It is well known that the ether-lead soap method does not give a complete separation of the saturated and unsaturated fatty acids, and this probably explains why the saturated fraction, the ether-insoluble lead soaps, still retained about one fourth of its inhibiting action, whereas the unsaturated fraction, the ether-soluble lead soap, now caused complete inhibition.

During the progress of the work we obtained three of Kahlbaum's supposedly pure soaps, sodium oleate, sodium palmitate, and sodium stearate. The results obtained with these three soaps and with one prepared by ourselves from olive oil are shown in text-figure 3. The iodin value of the linseed oil soap prepared by us was 90.

Our previous experiments indicated that the soaps of all the unsaturated fatty acids acted as inhibiting agents. We were therefore

surprised to note that the Kahlbaum sodium oleate was inactive. The solution of the problem was soon found, the sodium oleate had no iodin value; in other words, the oleic acid, through age or some other cause, had become saturated. The sodium soaps of palmitic and stearic acids, as we had anticipated, had no influence on either trypsin or leucoprotease.

All our experiments so far indicate that the inhibiting agents present in the soaps are the unsaturated fatty acids. The question therefore naturally presents itself: Can we by a saturation of these



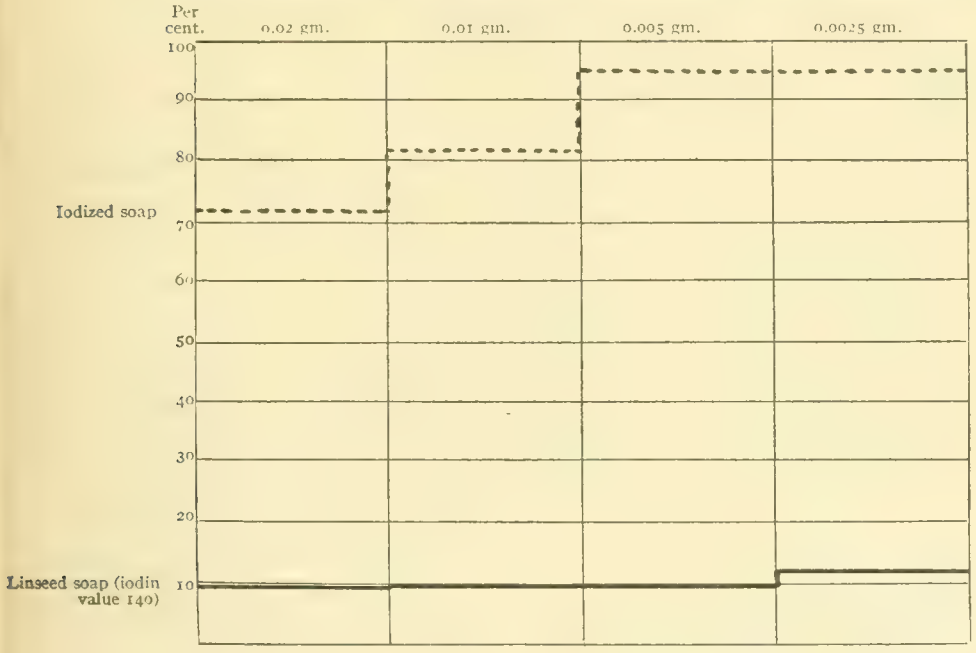
TEXT-FIG. 3. Effect of sodium oleate, sodium stearate, and sodium palmitate on tryptic digestion.

free bonds with iodin, for instance, destroy the inhibiting action and cause them to act like the soaps prepared with stearic, palmitic, and other saturated acids?

The results of one of the experiments made to enlighten us on this point are given in text-figure 4. This experiment was conducted with the ether-soluble lead soap fraction. To five cubic centimeters of a 1 per cent. solution of the soap were added 0.05 of a gram of iodin and a few crystals of iodide of potassium, and the mixture was permitted to stand over night. The following morning it was shaken repeatedly with chloroform, until fresh portions of the

latter remained clear, showing that there was no more free iodin. Text-figure 4 shows the action of the unsaturated soap before and after it was treated with iodin. Repeated tests had already shown that the small amount of iodin which might still remain would have a slight inhibiting action. This would explain why the soap still had some inhibiting action, though it may also be due to the fact that the acids were not completely saturated. The results of saturating the free carbon bonds are so obvious as to call for no comment, and also explain why the commercial sodium oleate was inactive.

Similar experiments made with sodium soaps of unsaturated fatty acids obtained from other sources indicated that the inhibiting



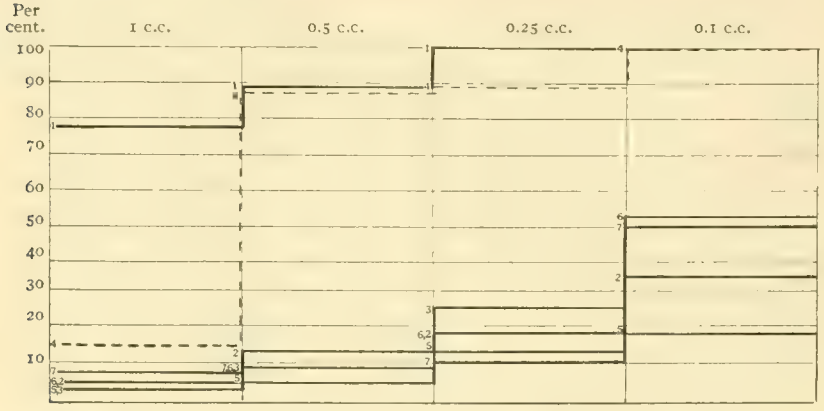
TEXT-FIG. 4. Effect of saturation of unsaturated linseed soaps with iodin.

action was due to the degree of unsaturation and that it could be almost entirely removed by saturation with iodin.

Our next experiments were made to determine whether the activity of the various soaps was dependent upon the number of unsatu-

rated bonds, as determined by their iodin values, or whether certain ones containing an equal number of unsaturated bonds were more active.

In this experiment soaps prepared from linseed oil, hempseed oil, castor oil, olive oil, croton oil, and cod-liver oil were used. After their respective iodin values had been determined, they were made up in such strengths that one cubic centimeter of each had the same iodin value; thus croton oil soap having less than half the value of the linseed oil soap was made up twice as strong. Text-figure 5



1 = saturated linseed soap.	Iodin value 15.	Solution 5.0	per cent.
2 = sodium oleate.	Iodin value 53.	Solution 1.9	per cent.
3 = croton soap.	Iodin value 55.	Solution 1.8	per cent.
4 = castor oil soap.	Iodin value 60.	Solution 1.7	per cent.
5 = hempseed soap.	Iodin value 100.	Solution 1.0	per cent.
6 = cod-liver oil soap.	Iodin value 90.	Solution 1.1	per cent.
7 = linseed soap.	Iodin value 135.	Solution 0.74	per cent.

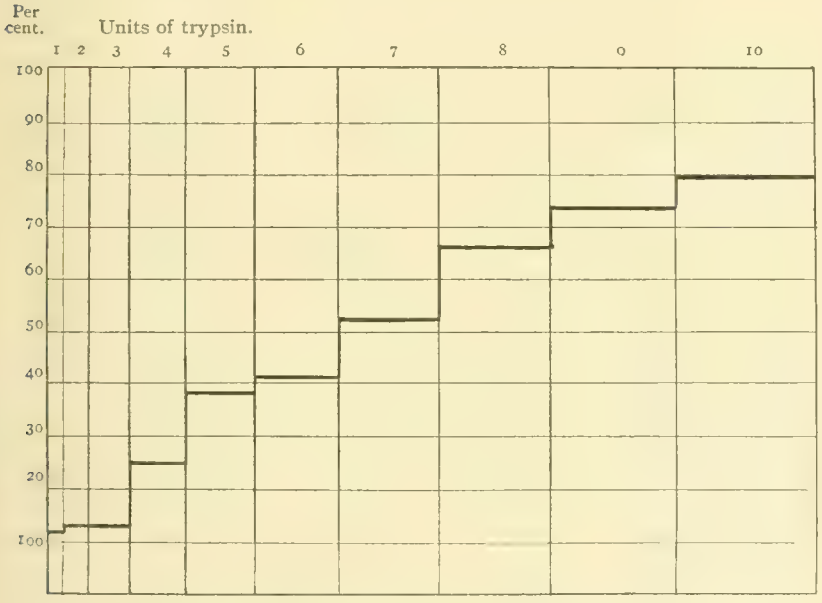
Text-Fig. 5. Relation of iodin value to inhibiting action of soaps on tryptic activity.

gives the results of this experiment. The lines shown on the chart indicate, with one exception, that the activity of the soaps is in proportion to their iodin values.

Here we have the first indication that the unsaturated fatty acids are not equally active as inhibiting agents, for the soap prepared from castor oil appears to be considerably less active. The inhibition obtained with one cubic centimeter was almost as great as that noted with the other soaps, but subsequent dilution proved it to be

much less active. With the exception of that prepared from linseed oil, the soaps used in this experiment were not obtained by the ether-lead soap method, and so their iodin values were low. It is possible that some of the unsaturated fatty acids are much more active than others in this respect. In fact, a part of our recent work indicates that those obtained from a certain source are much more active than any of those we have just discussed.

In the preceding experiments it has been demonstrated that 0.005 of a gram of the linseed oil soap was sufficient to prevent the action of the standard amount of trypsin. It was also found, in the standard proportions of ferment and substrate used, that the inhibiting action was still present in tubes containing much smaller amounts. In fact complete digestion was first obtained in the tube containing



TEXT-FIG. 6. Effect of increasing amounts of trypsin with single unit of soap.

0.0001 of a gram. In our next experiment we wished to find out if the smallest amount of soap producing nearly complete inhibition with the standard amounts of trypsin and casein would have any influence on larger amounts of the ferment. Text-figure 6 shows

the influence of the soap on increasing amounts of the ferment. 0.1 of a cubic centimeter of the ferment solution caused complete digestion in the control tube. The demonstration that such a small amount of soap, 0.005 of a gram, was able to affect materially the action of ten times the standard amount of ferment, indicates the great activity of these substances as inhibiting agents.

In the previous experiments we have shown that the unsaturated fatty acids inhibit proteolysis in an alkaline medium and that the degree of inhibition bears a close relation to their iodin values, but these experiments do not give us much information as to how the inhibition is effected. Is it due to a direct and permanent binding of the ferment with the unsaturated fatty acids, or to simple inhibition of ferment action, the ferment itself not being destroyed? If there is a true binding it would be difficult to extract the ferment after incubating with soap, but it might be done if the inhibition were due to some other cause.

Our next experiment was planned with the hope that some information might be obtained as to the nature of the inhibiting action. In this experiment the soaps of the saturated and unsaturated acids separated by the ether-lead soap method were mixed with the ferment and incubated for thirty minutes. The mixtures were then made slightly acid with hydrochloric acid and extracted with ether. After having removed the ether, the solution was made slightly alkaline, the casein added and again incubated. One control tube of ferment without soap was treated in the same manner so as to rule out the effect of the acid and ether. In this experiment we wished to find out if the soap simply inhibited the action of the ferment or destroyed it. Table I gives the results of this experiment.

TABLE I.

	Trypsin.	1 per cent. unsaturated linseed soap, iodin value 130.	1 per cent. saturated linseed soap, iodin value 15.	Sodium chloride solution.	1 per cent. casein.	Total incoagulable nitrogen.	Digestion.
1	0	0	0	3.0 c.c.	2.0 c.c.	0.04 mg.	2 %
2	0.2 c.c.	0	0	2.8 c.c.	2.0 c.c.	1.66 mg.	100 %
3	0.2 c.c.	0.5 c.c.	0	2.3 c.c.	2.0 c.c.	0.10 mg.	6 %
4	0.2 c.c.	0	0.5 c.c.	2.3 c.c.	2.0 c.c.	1.30 mg.	80 %
5	0.2 c.c.	0	0	2.8 c.c.	2.0 c.c.	1.50 mg.	90 %
6	0.2 c.c.	0.5 c.c.	0	2.3 c.c.	2.0 c.c.	0.20 mg.	12 %
7	0.2 c.c.		0.5 c.c.	2.3 c.c.	2.0 c.c.	1.60 mg.	100 %

The first four tubes in the table are the untreated controls. The first tube shows the percentage of incoagulable nitrogen in the casein alone; the second, the total nitrogen in complete digestion; the third and fourth show the influence of the saturated and unsaturated soaps on tryptic activity. Tubes 5, 6, and 7 show the influence of acid and ether extraction. Tube 5 contained no soap but was treated in the same way as Nos. 6 and 7 to make certain that the method used had not destroyed the ferment. Tube 6 containing the unsaturated soap shows that the ferment action is almost inhibited, while in tube 7 containing the saturated soap there is no inhibition. In other words, incubating the ferment with soaps of the unsaturated fatty acids for thirty minutes causes its destruction, the removal of the acids after incubation not being sufficient to reactivate the ferment, while the ferment present in the tube containing the saturated acid, though treated in the same manner, is not destroyed.

The results obtained in this preliminary study of the action of the soaps prepared from the unsaturated fatty acids suggest that they may play an important part in the body, particularly in certain pathological processes. Whether this inhibition of ferment action is due to a binding of the ferment with the unsaturated carbon atoms, or whether to some physical condition brought about by the presence of the soap, remains to be determined. If due to some physical condition brought about by the presence of the soap, similar results ought to be obtained with the saturated fatty acids, but we have demonstrated that the latter are inactive in this respect. In addition, the experiments conducted with soaps saturated with iodin show that the presence of unsaturated bonds are necessary for the development of this property. Further proof that this action is due to the degree of unsaturation is afforded by text-figure 5, which indicates that the activity of the soaps is proportionate to their iodin value, while the soaps having no iodin value, sodium stearate and sodium palmitate, are inactive.

SUMMARY.

1. Sodium soaps prepared from olive oil, croton oil, cod-liver oil, linseed oil, etc., have the property of inhibiting the action of trypsin and leucoprotease.

2. The activity of these soaps is dependent upon the degree of unsaturation of the fatty acids and is in proportion to their iodin value.

3. Saturation of the acids with a halogen (iodin) causes a loss of this property.

4. Soaps of the saturated fatty acids tested do not have this influence on ferments.

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FERMENT INHIBITING SUBSTANCES IN TUBERCLE BACILLI.

Studies on Ferment Action. XI.*

By JAMES W. JOBLING, M.D., and WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

A great deal of work has been done in the attempt to explain the processes that lead to the production of caseation in tuberculosis. By some it has been ascribed to anemia, the tubercle being avascular, and by others to the action of toxins derived from the tubercle bacilli.

Auclair (1) believes that the caseation is due to specific toxins of the nature of fat, which are soluble in ether, chloroform, benzine, and xylol. He treated tubercle bacilli with one or more of the above agents, evaporated the solvents, and suspended the extracted substances in water. These emulsions, when injected into the subcutaneous tissues of animals, produced typical caseous abscesses. When injected into the trachea of guinea pigs, caseous areas appeared in the lungs.

Schmoll (2) analyzed caseous material and found it almost free from proteoses, which indicates that autolysis is slight; but he does not explain the lack of autolysis. The work of Schmoll has been confirmed by others, and we have obtained similar results.

Anemia due to the occlusion of the blood vessels may be an important factor in causing caseation in the chronic forms of tuberculosis where the areas are surrounded by a layer of connective tissue. However, in acute caseous pneumonia in which there is a catarrhal exudate rapidly becoming caseous, some other explanation appears to be necessary. In such an exudate there are numerous cells known to contain ferments, yet these die and disintegrate, and, as far as can be determined, the ferments remain inactive. We must therefore look for some other factor to explain the process of caseation, and this we believe we have found in the tubercle bacillus, a ferment-inhibiting substance. We observed two years ago that the

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antitryptic action of sera was lost or greatly decreased when the sera were preserved with chloroform. This observation suggested to us the possibility that the antiferment properties of the blood were due to substances of the nature of lipoids, and the lack of autolysis in caseation and in anemic infarcts to substances of a similar nature.

With this hypothesis we decided to study the influence of the ether-soluble substances of tubercle bacilli on proteolysis. It has been shown by numerous investigators that the ether-soluble substances constitute 25 to 35 per cent. by weight of the bacilli, and of these extracts about 17 per cent. are composed of fatty acids. Both human and bovine tubercle bacilli were used in the work.[1]

Our first experiments were conducted with ether- and alcohol-soluble substances obtained from the tubercle bacilli. After the solvent had been evaporated, the material remaining was taken up in methyl or ethyl alcohol and suspended in a 0.9 per cent. solution of sodium chloride. In some experiments the emulsion was used just as prepared; in others the alcohol was first evaporated at low temperatures. The emulsion, in various dilutions, was then mixed with trypsin, incubated for thirty minutes, and the casein added. As far as we could determine there was no inhibition of ferment action.

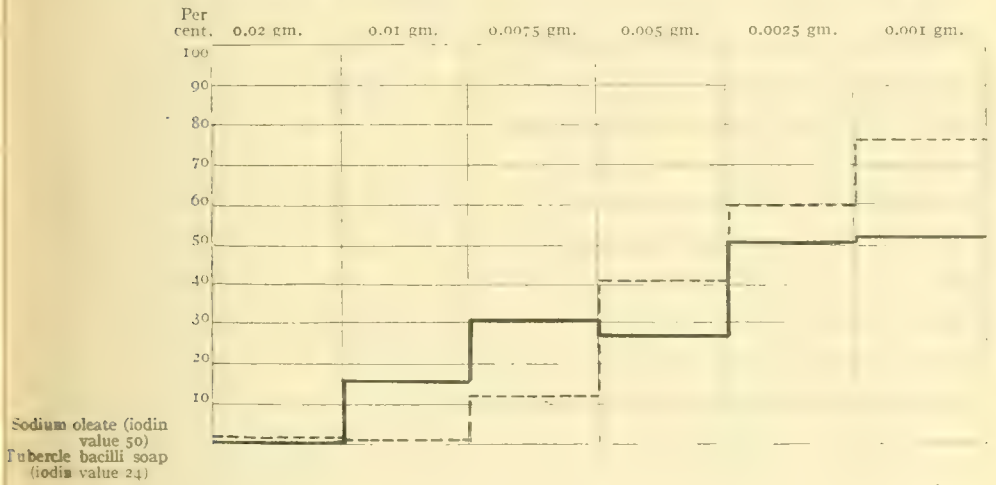
In the preliminary experiments we used the Fuld-Gross technique, but, for reasons given in our preceding paper (3), we soon discarded it in favor of the following. After incubating the mixtures for a certain period of time, they were acidified with a solution containing 10 per cent. acetic acid and 20 per cent. sodium chloride. The tubes were then placed in boiling water for five to ten minutes, filtered through kaolin to remove the coagulated protein, and the incoagulable nitrogen was determined according to the method recommended by Folin (4).

This experiment failed to demonstrate the presence of an inhibiting agent. This may have been due to the agent being in an emulsion and not a solution, thus preventing its coming into intimate con-

[1] We wish to thank Dr. Hitchens, of Glenolden, Pa., and Dr. Grund, of the New York Board of Health, for supplying us with large amounts of human and bovine tubercle bacilli.

tact with the ferment. We then fractionated the extracted material so that at least a part, the fatty acids, might be prepared in such a manner that they could be brought into solution. The material was dissolved in ether and precipitated with acetone. The acetone fraction was evaporated to dryness on the water bath and the resulting substance saponified with alcoholic potash. The soap was dissolved in water and repeatedly extracted with petroleum ether in order to remove any unsaponifiable matter. The acids were then liberated with hydrochloric acid, taken up with ether, washed with water, and resaponified.

The ferments were prepared and standardized according to the

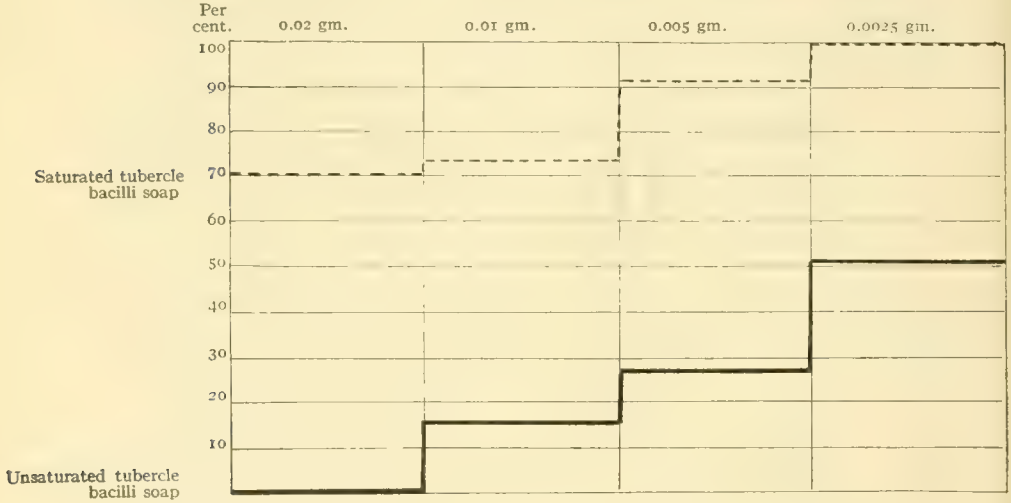


TEXT-FIG. 1. Effect of soaps of tubercle bacilli and sodium oleate on tryptic digestion.

method described in our previous paper (5). The soaps, in 1 per cent. solutions, were mixed with trypsin in varying amounts, incubated for thirty minutes, and the casein was then added. After the final incubation of two hours, the coagulable protein was removed and the incoagulable nitrogen determined. Text-figure 1 shows the results obtained with the soap prepared from extractives of the tubercle bacilli. A soap prepared from olive oil was used as a control. The dotted and heavy black lines indicate the percentage of digestion present in the tubes containing soaps as compared with the controls containing trypsin and casein alone.

The results of this experiment show that in the final dilutions the soap prepared from tubercle bacilli is more active as an inhibiting agent than the one prepared from olive oil. The iodin value of the soap prepared from the tubercle bacilli was 24, of the olive oil soap 50. The relation that the iodin value of the soaps bears to their activity as inhibiting agents was discussed in our previous report. It will be dealt with here in relation to the one prepared from tubercle bacilli.

In the previous paper (5) on the influence of soaps of the fatty acids on inhibition of ferment action, we demonstrated that the unsaturated fatty acids were the active agents, while the saturated fatty acids exerted but little if any inhibiting action. We were unable to obtain a complete separation of the saturated from the unsaturated acids and so could not remove entirely the inhibiting



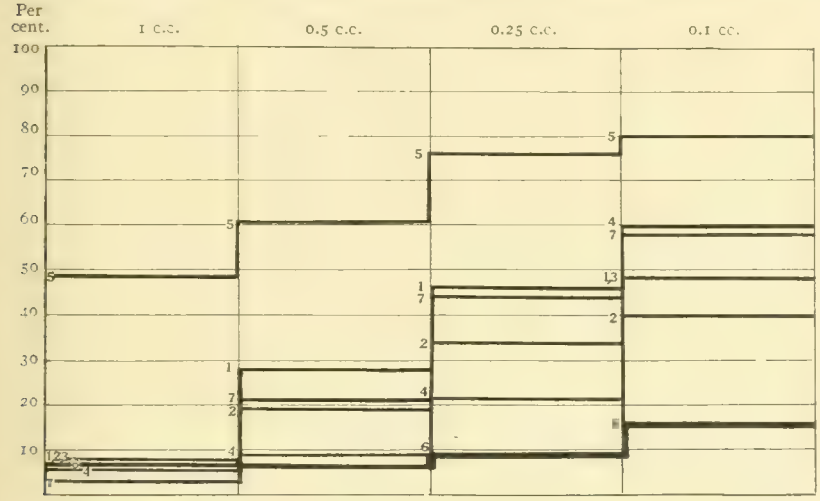
TEXT-FIG. 2. Effect of saturated and unsaturated soaps of tubercle bacilli on tryptic digestion.

action, but the differences in their activity following their partial separation by the ether-lead soap method were so striking as to be conclusive. The soaps of the saturated fatty acids tested, sodium stearate and sodium palmitate, were inactive.

We undertook to determine if the same is true of the soaps pre-

pared from tubercle bacilli. Lead soaps were prepared according to the usual method. The acids were set free with hydrochloric acid, washed, resaponified with sodium alcoholate, and evaporated to dryness. Text-figure 2 gives the results obtained with soaps prepared from saturated and unsaturated fatty acids.

The results obtained with the ether-soluble and insoluble fractions are as conclusive as those described in the preceding report for other soaps. The ether-lead soap method does not give a complete separation of the saturated and unsaturated fatty acids, but the separation is sufficient to demonstrate which is the active fraction.



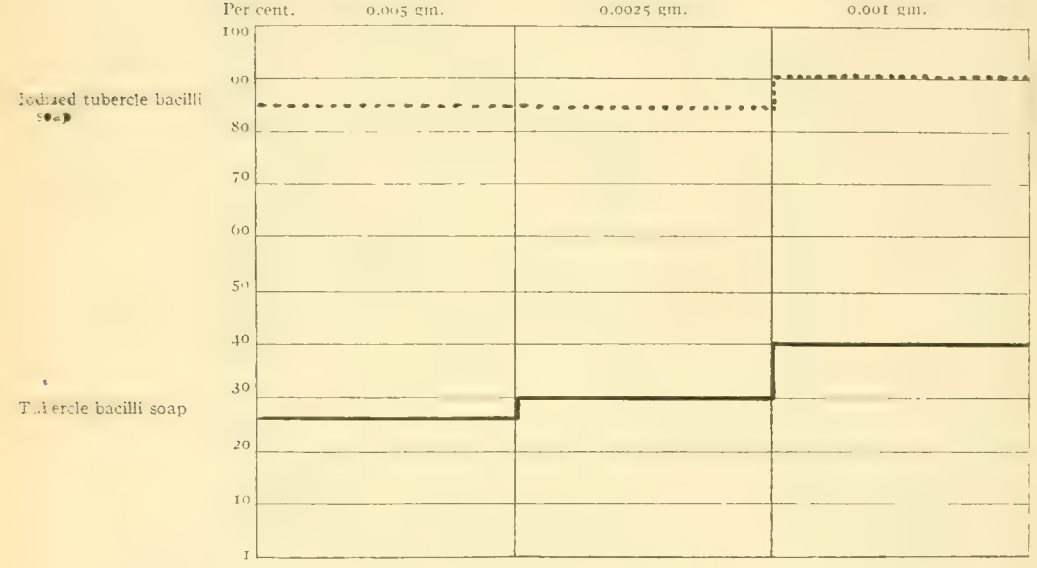
1 = hempseed soap.	Iodin value 100.	Solution 1.0 per cent.
2 = linseed soap.	Iodin value 100.	Solution 1.0 per cent.
3 = croton soap.	Iodin value 61.	Solution 1.6 per cent.
4 = olive oil soap.	Iodin value 44.	Solution 2.2 per cent.
5 = castor oil soap.	Iodin value 35.	Solution 3.0 per cent.
6 = tubercle bacilli soap.	Iodin value 25.	Solution 4.0 per cent.
7 = cod-liver oil soap.	Iodin value 92.	Solution 1.1 per cent.

TEXT-FIG. 3. Effect of soaps of tubercle bacilli and various other soaps on tryptic digestion.

We reported previously that, with one exception, the activity of the soaps of the unsaturated fatty acids as inhibiting agents was proportionate to their iodin value. We did not include in the experiment described the soap prepared from the extractives of

the tubercle bacilli. This is shown in text-figure 3 in comparison with the other soaps which we have been using. After the iodin value of the respective soaps had been determined, they were made up in such strengths that one cubic centimeter of each had approximately the same iodin value.

A study of text-figure 3 shows that of all the soaps investigated the one prepared from tubercle bacilli is the most active in preventing enzyme action. We had already observed that it was as active as the other soaps in 1 per cent. solutions and were not surprised to find it more so when made up according to its iodin value.



TEXT-FIG. 4. Effect of saturation of unsaturated soaps of tubercle bacilli with iodin.

The method used by us in extracting the acids from the bacilli and in preparing the soap does not give any indication of the quantity originally present. On the contrary, it offered every opportunity for the unsaturated acids to become saturated, thus lowering its iodin value and reducing its activity as an inhibiting agent.

In the previous report we showed that the activity of the soaps of the unsaturated fatty acids could be destroyed by saturation with iodin. The soaps so treated did not become entirely inactive.

but this may have been due either to the presence of some free iodin or to incomplete saturation. It is of importance to determine if the soaps prepared from tubercle bacilli are similarly affected when treated with iodin. To five cubic centimeters of a 1 per cent. solution of the soap was added 0.05 of a gram of iodin, and a few crystals of iodide of potassium, and the mixture was permitted to stand over night. The following morning it was shaken repeatedly with chloroform, until fresh portions of the latter remained clear, showing that there was no more free iodin. Text-figure 4 shows the effect of saturating the soap with iodin.

The soap used in this experiment was old and therefore not very active, but the effect of treatment with iodin was evident. The experiment was repeated several times and a similar result obtained in each instance.

Numerous chemicals have been studied in the attempt to find one that will be effective as a therapeutic agent in tuberculosis. Such an agent, in order to be of value, must combine with some constituent of the tubercle bacillus, and our experiments indicate that iodin as a component of the chemical substance may be the means of bringing about such a combination.

We do not know the nature of the unsaturated fatty acids exerting the inhibiting action, but their isolation and identification and further work on their inhibiting action are now under way.

Wells (6) obtained an average of 0.001 of a gram of fatty acids to one gram of caseous matter, which indicates the small amount of soap present in this substance. This finding has little bearing on the relation of the fatty acids of tubercle bacilli to caseation, as the influence of the soaps depends on the amount of ferment present and not on the quantity of protein. That small amounts of the soap are capable of inhibiting enzyme action is shown in text-figure 1, where it was found that in the tube containing 0.001 of a gram of soap the digestion was only half that obtained in the control containing no soap. The influence of soaps as inhibiting agents was well shown in our previous report in which it was demonstrated that the smallest quantity completely inhibiting the standard amount of trypsin materially influences the action of ten times that amount of ferment. In

addition, our experiments indicate that the ferments are destroyed as a result of combining with the soaps.

The soaps are probably formed from the neutral fats of the bacilli. The lipases hydrolyze the fats and the acids are then saponified. In partial support of this view we have the work of Klotz (7) who demonstrated lipases in tuberculous pus, and of Bergel (8) who states that the ferment is present in the lymphoid cells of tubercles, while Corper (9) found lipases in tubercle bacilli.

The demonstration that tubercle bacilli contain ferment-inhibiting substances brings us one step nearer a rational explanation of caseation in tuberculosis. The subject will be discussed more fully in a subsequent report.

CONCLUSIONS.

1. Tubercle bacilli contain unsaturated fatty acids which, when saponified, have the property of inhibiting the action of trypsin and leucoprotease.

2. In proportion to their iodin value these soaps are more active as inhibiting agents than the soaps prepared from linseed, olive, and cod-liver oils.

3. The activity of the soaps is dependent on the presence of unsaturated carbon bonds.

4. Saturation of the soaps with iodin destroys their inhibiting action.

5. Soaps probably play an important part in the production of the condition known as caseation in tuberculosis.

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A STUDY OF THE FERMENTS AND FERMENT-INHIBITING SUBSTANCES IN TUBERCULOUS CASEOUS MATERIAL.

STUDIES ON FERMENT ACTION. XII.*

BY JAMES W. JOBLING, M.D., AND WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

In a previous paper (1) we reported that sodium soaps prepared from ether-soluble extractives of tubercle bacilli inhibit the action of trypsin and leucoprotease. The inhibiting action was found to depend on the presence of soaps of the unsaturated fatty acids, and could be destroyed by saturating the acids with iodin.

The results of this study appear to have a direct bearing on the development of caseation in tuberculosis. Caseation in tuberculosis is a form of coagulation necrosis in which the dead tissues rarely undergo autolysis, except as a result of secondary infection. Syphilis is the only infectious disease presenting a similar condition. In other instances of coagulation necrosis the dead tissues are soon removed by means of autolysis and phagocytosis. It appears, then, that substances having the property of preventing autolysis must be present in syphilitic and tuberculous tissues.

This view is confirmed by the work of Schmoll (2) who was unable to find any evidences of autolysis in caseous material. The results obtained by Auclair (3) can be explained in a similar manner. Auclair believes that caseation is due to specific toxins of the nature of fats which are soluble in ether, chloroform, and benzin. These extracts suspended in water produced typical caseous abscesses when injected into the subcutaneous tissues of animals, and caseous areas in the lungs when injected into the trachea of guinea pigs. No doubt the conditions observed by Auclair closely simulated caseous areas, but we doubt if they were due to the presence of specific toxins. It is more probable that his injections caused the usual inflammatory exudate which assumed the character of the caseous matter owing to the failure of autolysis.

The lack of autolysis in caseous material is similar to that observed in non-

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infected anemic infarcts. In the latter condition the dead tissue is finally removed by the phagocytic leucocytes which invade it from the surrounding tissues. The leucocytes also liberate ferments which aid in causing solution and absorption of the dead tissue. It is only in the large infarcts that softening occurs, and this is in the central part of the mass, while the periphery remains firm. Wiener (4) found that the autolytic enzymes act best in an acid medium, and Wells (5) believes that this explains the central softening in large infarcts. According to Wells, the acids at the periphery are neutralized by the blood plasma, so that autolysis is active only at the center.

Anemia, due to occlusion of the blood vessels, may be an important factor in causing caseation in the chronic forms of tuberculosis. However, in acute caseous pneumonia some other explanation seems necessary. Here we do not have the connective tissue reaction seen in the chronic forms of the disease, and we must therefore look for some other cause to explain the conditions present.

In a previous report (6) we showed that tubercle bacilli contain unsaturated fatty acids which, when saponified, are able to inhibit enzyme action. We believe that this observation explains the lack of autolysis in caseous matter, but it was considered necessary to make a careful study of caseous material in order to find out if ferments and ferment-inhibiting substances were present.

Schmoll's (7) failure to find evidences of autolysis in the caseous material from lymph glands indicates that ferment action has been inhibited from the start. This assumption is based not only upon Schmoll's work, but also upon our observation (8) that trypsin is destroyed when incubated for thirty minutes with soaps of the unsaturated fatty acids. If this view is correct, failure of proteolysis is due to destruction of the ferments by the soaps, but we must also consider the possibility of undestroyed ferments being present, though inactive, owing to the presence of inhibiting substances. It is unlikely that free ferments should be present in the caseous material obtained from chronic tuberculous lymph glands, but they may be present in a rapidly progressing caseous pneumonia with a marked inflammatory exudate. In the latter condition it is impossible to separate the caseous material from the inflammatory exudate, and so extracts of the caseous material will probably show the presence of some undestroyed ferments.

The caseous matter used in this study was obtained from tuberculous lymph glands and from several cases of rapidly progressing

caseous pneumonia.[1] The method used to determine the extent of digestion was that described in our previous reports. The mixture was made definitely acid with a solution composed of 10 per cent. acetic acid and 20 per cent. sodium chloride. The tubes were then placed in boiling water for five to ten minutes to coagulate the protein and the contents afterwards filtered through kaolin. The total incoagulable nitrogen was determined according to the method recommended by Folin.

CASEOUS LYMPH GLANDS.

The caseous matter derived from the lymph glands was spread in thin layers on glass plates and dried at temperatures below 45° C. After being dried the material was ground in a mortar to a fine powder. For the experimental work the emulsions were prepared in the proportions of one gram of caseous matter to 100 cubic centimeters of water. The material was examined to find out if ferments and ferment-inhibiting substances were present and also for evidences of autolysis. To determine the extent of autolysis a flask containing fifty cubic centimeters of the emulsion was placed in the incubator and left there for five days. Every day five cubic centimeters were removed and the amount of incoagulable nitrogen was determined. The incoagulable nitrogen obtained in five cubic centimeters of the emulsion on the fifth day was practically the same as that obtained on the first day. The results of this experiment indicate that either ferments are not present in the caseous matter, or their activity is suppressed. The freshly prepared emulsion was neutral in reaction, but became slightly acid after the second day. This is important as in the beginning the reaction was favorable to the ferments active in an alkaline or neutral reaction, and after the second day it was favorable to those active in an acid reaction, yet autolysis did not occur.

We obtained somewhat different results in a similar experiment conducted with caseous material from a lymph gland which had become secondarily infected. Autolysis occurred in a more marked

[1] We wish to thank Dr. F. Mathews of St. Mary's Free Hospital, Dr. Larkin of the New York Hospital, and Dr. Lamb of the Presbyterian Hospital for their kindness in furnishing us with material.

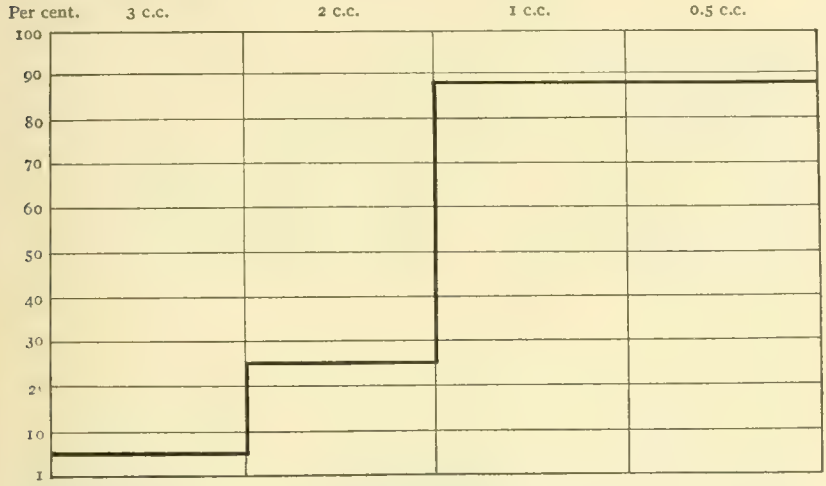
degree in an alkaline reaction, but was much more active in an acid reaction. In no instance have we been able to obtain evidences of autolysis in emulsions of uninfected caseous matter of lymph glands, either in acid or alkaline reactions. The lack of autolysis in the caseous material might have been due to the presence of soaps, and we therefore attempted to rule out this factor.

Two methods were used to determine the presence of undestroyed ferments. In the first the whole emulsion was treated with an excess of alcohol to which a small amount of hydrochloric acid had been added. Treated in this manner the ferments and protein were precipitated, and the fatty acids liberated by the hydrochloric acid were made soluble in the acid alcohol. The precipitate was then washed with alcohol and ether, and dried. The powder thus obtained was made into an emulsion with water, a portion made acid and another alkaline, and placed in the incubator for several days. The presence of ferments would have been shown by the degree of autolysis that occurred. In the second method the emulsion was first incubated for twenty-four hours in an alkaline solution in order to get the ferments and soap into solution. It was then centrifuged and the supernatant fluid treated as was the whole emulsion. In this instance the powder was made into an emulsion and tested against casein. In neither case were we able to demonstrate the presence of ferments.

We believe that the results of these experiments justify us in concluding that undestroyed ferments are rarely present in caseous lymph glands which have not become secondarily infected.

As practically all cells contain intracellular ferments, we must assume that substances were present which prevented autolysis. Our previous work indicated that these substances were probably soaps, therefore our next experiments were planned to see if this was the case. A fresh 1 per cent. emulsion was prepared and kept at room temperature for several hours. During this period it was shaken frequently. It was then centrifuged until it became clear. The supernatant fluid was now removed, made strongly alkaline with sodium hydrate, and the flask containing it placed in boiling water for several minutes. The fatty acids were then liberated with hydrochloric acid, taken up in ether, and resaponified.

The last step was repeated in order to free the soaps from unsaponifiable matter. The soaps were then tested for their enzyme-inhibiting action. They were mixed in various dilutions with trypsin and placed in the incubator at 37 C. for thirty minutes. The casein was then added and the mixture placed in the incubator again for about two hours. After the final incubation the total incoagulable nitrogen was determined according to Folin's method. Text-



TEXT-FIG. 1. Effect of the saponified extract from caseous lymph glands on tryptic digestion.

figure 1 shows the influence of soaps obtained from the caseous matter of lymph glands on the activity of trypsin.

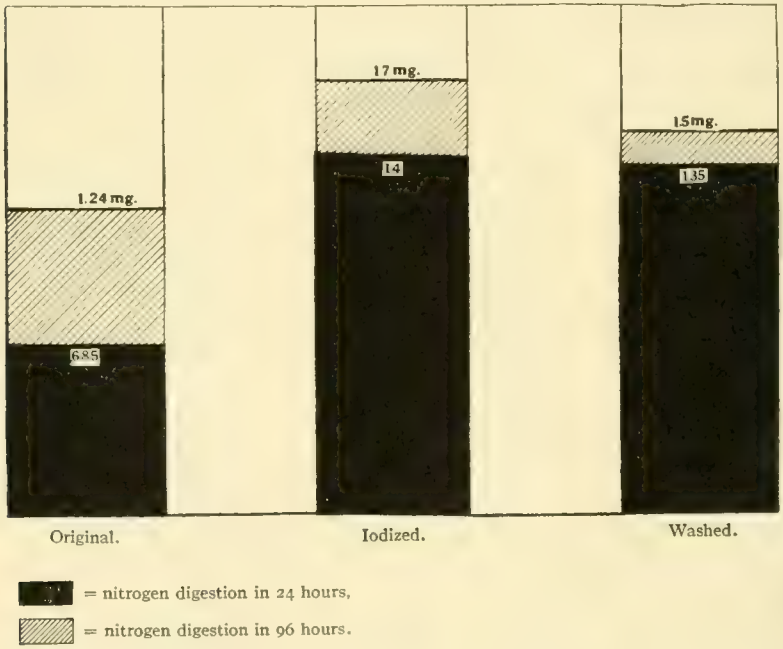
The soaps had been dissolved in sufficient water so that one cubic centimeter of the solution represented the amount of soap contained in one cubic centimeter of the original 1 per cent. emulsion. Three cubic centimeters of the emulsion, representing the soap contained in 0.03 of a gram of caseous matter, was sufficient to inhibit the action of the ferment. In the tube containing two cubic centimeters the digestion was only 25 per cent. of that obtained in the control tube containing no soaps.

The results obtained with the caseous material taken from a lymph gland which had become secondarily infected differ from those just described. Similar experiments were conducted with

this material, which was semifluid in consistence, and ferments active in alkaline and acid reactions were demonstrated. While autolysis occurred in both acid and alkaline reactions, it was more marked in the acid reaction, indicating that soaps were still present.

With this possibility in mind we decided to study the influence of iodin on the whole emulsion to see if it would accelerate autolysis. We have already shown (9) that treating the soaps of the unsaturated fatty acids with iodin destroys their ferment-inhibiting action, and it is therefore of interest to determine if similar results can be obtained with the native mixtures of protein and soaps.

The emulsion was divided into two portions, one of which was used as a control. To the other was added a mixture of iodin and iodide of potassium. The mixture was allowed to stand for several hours, and the excess of iodin was then removed by repeated extractions with chloroform. A third flask containing the washed precipitate was also used. To each of these flasks trypsin was



TEXT-FIG. 2. Effect of iodin on ferment-inhibiting substances present in caseous matter of lymph glands.

added. Text-figure 2 shows the amount of incoagulable nitrogen in two cubic centimeters of the emulsion after twenty-four and ninety-six hours' incubation at 37° C. The first column shows the amount of incoagulable nitrogen obtained after digesting the untreated emulsion with trypsin. The second column represents the amount of nitrogen obtained by digesting the iodized fraction, and the third the action of trypsin on the precipitate which had been freed from soaps by extraction with water and repeated washings.

A study of this chart shows that in the first tube soaps were not present in sufficient quantity to inhibit the action of the trypsin, but that digestion in a similar fraction treated with iodin was as great at the end of twenty-four hours as that obtained in the first tube after ninety-six hours. In the third tube the soaps had evidently been removed to a large extent, though digestion in the last seventy-two hours was not equal to that observed in the iodized fraction.

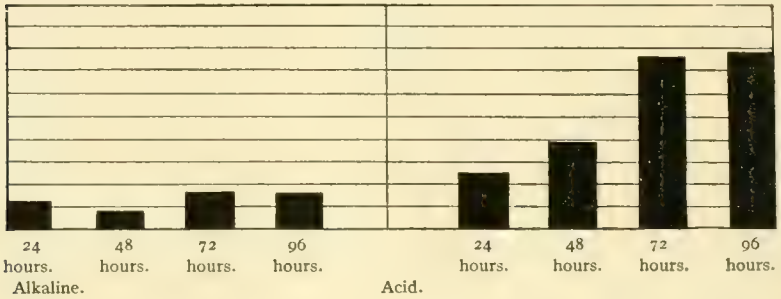
CASEOUS PNEUMONIA.

The affected areas in the caseous lungs were freed as much as possible from the less involved lung tissue, cut up in a meat machine, and dried. When dry the material was ground to a fine powder in a mortar. We anticipated somewhat different results in these experiments from those obtained with caseous material from lymph glands, owing to the difficulty of freeing the caseous material from the inflammatory exudate. In the former case there is usually no inflammatory exudate and therefore no extraneous ferments, while in the material from caseous pneumonia these ferments must be considered.

Several specimens of this kind were examined, and practically similar results were obtained with all. As in the experiments conducted with the caseous matter of lymph glands, the emulsion was made in the proportion of one gram of the dried material to one hundred cubic centimeters of water.

In the first experiment we wished to determine the extent of autolysis. Two flasks containing twenty-five cubic centimeters each of the emulsion were incubated for several days at 37° C. In order to determine the type of ferments present, the contents of one of

the flasks was made slightly acid in reaction, and the other slightly alkaline. Text-figure 3 shows the result of this experiment. In studying this chart it should be remembered that soaps are dissociated in an acid reaction, and are therefore inactive as inhibiting agents. Each line on the chart represents 0.1 of a milligram of nitrogen, and the black columns indicate the incoagulable nitrogen in



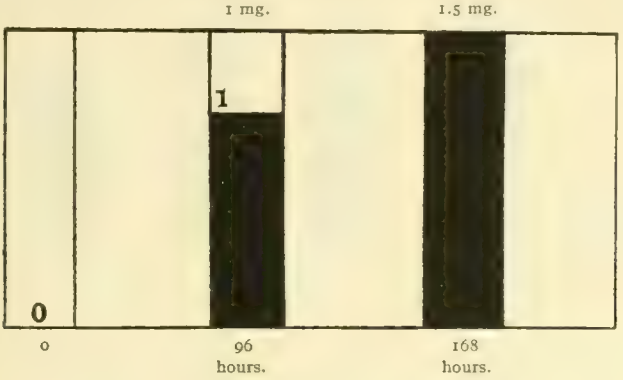
TEXT-FIG. 3. Autolysis of caseous material in acid and alkaline reactions.

two cubic centimeters of the emulsion. The chart shows the differences obtained in the presence of acids and alkalies. Inhibiting substances were present in sufficient quantity to prevent the action of ferments active in an alkaline reaction, but they were not effective when the reaction was made acid.

In some of our preliminary experiments we observed that autolysis occurred in certain of the emulsions which had been made slightly alkaline, and we were at a loss for the proper explanation as soaps were known to be present. We found later that in practically all such instances the reaction of the emulsion had become acid, thus throwing out the soaps and permitting the action of ferments active in an acid reaction. In the last experiment it was evident that ferments active in an acid reaction were present, but that those active in an alkaline reaction were absent or their activity was suppressed by the soaps. In the next experiment we wished to determine if ferments active in an alkaline reaction were also present in the caseous material.

The emulsion was treated with an excess of alcohol which had been made slightly acid. By this means we hoped to precipitate the ferments uninjured and at the same time to remove the soaps, or

rather the fatty acids, which, treated in this manner, are soluble in alcohol. The precipitate was removed by centrifugalization, washed with alcohol and ether, and dried. It was then brought up to the original volume with water, made slightly alkaline, and placed in the incubator for seven days. The results of this experiment are seen in text-figure 4. The columns represent the amount of incoag-



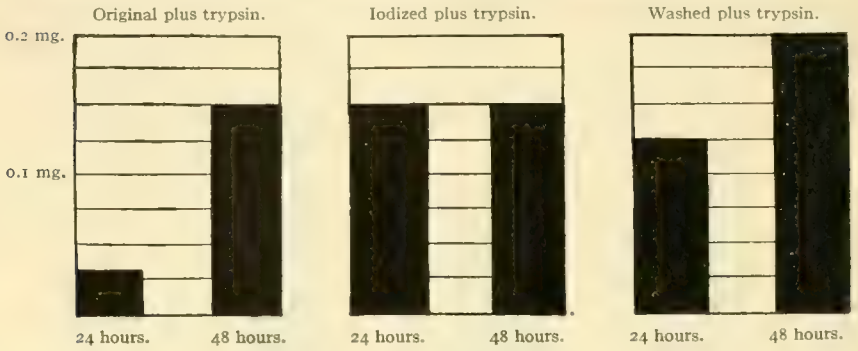
TEXT-FIG. 4. Autolysis of alcohol precipitate.

ulable nitrogen in two cubic centimeters of the mixture. The chart shows that ferments active in an alkaline reaction were present in the original material, but that their activity was inhibited by the soaps. When these were removed by the acid alcohol, the caseous matter underwent autolysis. It is unlikely that these tissues become acid during life, and so the lack of autolysis is easily explained.

Soaps were present in smaller amounts than in the caseous matter of lymph glands. This may be due to the fact that the rapidity of the process did not permit an accumulation of the soaps such as probably occurs in the chronic tuberculous lymph glands. Text-figure 3 shows that autolysis did not occur when the original emulsion was made slightly alkaline, though in other experiments in which the soaps were first removed, we demonstrated the presence of ferments active in an alkaline reaction. These results indicate a slight excess of soaps over the amount needed to prevent autolysis. This view is confirmed in the next experiment in which it is shown that with an increase of ferment autolysis ensues.

In our experiments with the caseous matter of lymph glands we

showed that previous treatment of the emulsion with iodin was effective in aiding the action of trypsin. We now made similar experiments with the pneumonic material, using the same technique. Text-figure 5 shows the results obtained. Two controls were used.



TEXT-FIG. 5. Effect of iodin on ferment-inhibiting substances in caseous pneumonia.

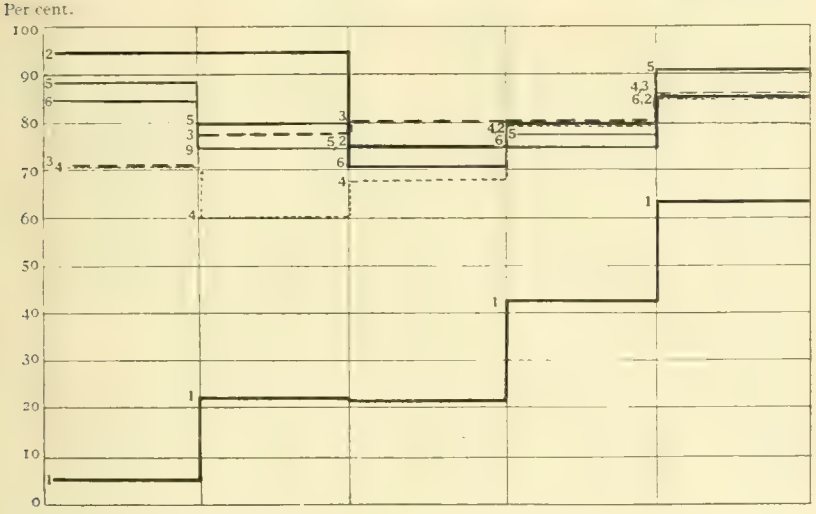
One control consisted of the untreated emulsion with trypsin, and the other of the washed precipitate with trypsin. This last control was used to see if all the soaps could be removed from the precipitate by repeated washings with water.

Here again we have evidence that previous treatment of the emulsion with iodin increased the activity of the trypsin. The amount of incoagulable nitrogen obtained after twenty-four hours' incubation of the iodized emulsion was much greater than that observed in the untreated fraction, and also greater than that obtained with the washed precipitate. It is probable that the washed precipitate was not entirely free of soaps. The amount of digestion observed after forty-eight hours in the untreated emulsion indicates that soaps were not present in large amounts. In a previous paper (9) we showed that soaps were able to inhibit completely the action of enzymes only in certain proportions, and that digestion occurred when enzymes were present in excess of this proportion. This is probably the explanation in the present instance, as the results show that soaps are present in comparatively small amounts in these pneumonic areas (text-figure 6).

In addition to the extractives of the tubercle bacilli which we have

been discussing, lipoids derived from dead cells are also present in caseous matter. Neumann (10) and others state that an alkaline solution of lecithin inhibits tryptic activity, but Meyer (11), using a 1 per cent. solution, was unable to confirm these observations.

In view of the uncertainty of the action of the lipoids we made a few experiments in order to determine their influence on enzyme activity. For these experiments lecithin, cuorin, and cholesterol were used. The solutions were prepared by mixing one gram of the lipoid with 100 cubic centimeters of a 1 per cent. solution of an inactive preparation of sodium oleate. This sodium oleate prepara-



1 = soaps of tubercle bacilli.
2 = sodium oleate (saturated).
3 = soaps of tubercle bacilli (from waxes).
4 = lecithin.
5 = cuorin.
6 = cholesterol.

TEXT-FIG. 6. Effect of soaps of tubercle bacilli and lipoids on tryptic digestion.

tion was one in which the acid had become saturated, thereby rendering it inactive as an inhibiting agent. By suspending the lipoids in an inactive soap solution we hoped to duplicate the physical condition present in the control containing the soap prepared from tubercle bacilli. Text-figure 6 gives the results obtained in one of the experiments.

In addition to the preparations mentioned above, there was also tested in this experiment a soap prepared by saponifying the acetone-insoluble fraction of the extractives of the tubercle bacilli. This soap is apparently much less active than that prepared from the acetone-soluble fraction.

All the lipoids when tested in this manner appear to have some inhibiting action, particularly lecithin which is the most active. The experiment indicates that lipoids in the tissues, particularly in the presence of soaps, may aid in preventing autolysis, but their activity is much less than that demonstrated for the soaps.

DISCUSSION.

We believe that we are now in a position to understand more clearly the processes leading to caseation in tuberculosis. With the lodging of the bacilli there is death of some of the cells due to the action of the toxins, and a proliferation of the neighboring cells. Under other conditions the death of a small number of cells would be followed at once by their removal, either through the action of phagocytes or by autolysis; but here the phagocytes are absent, and the action of the enzymes is prevented by the presence of the soaps of the unsaturated fatty acids derived from the tubercle bacilli. The anemia caused by the occlusion of the blood vessels plays an important part, as the lack of fluid prevents the dilution and subsequent absorption of the inhibiting substances. As the local process spreads slowly, the reaction at the periphery of the area prevents the entrance of fluids, and thus the caseous area gradually increases in size as the bacilli invade and cause the death of the surrounding tissues.

In conditions such as those present in caseous pneumonia, there is primarily an exudate composed of desquamated cells, leucocytes, and fibrin, which soon becomes caseous. Before caseation is complete in the zone of gelatinous pneumonia, there is not the anemia due to occlusion of vessels that is seen in the more chronic processes, and we must conclude that persistence of caseation is due to substances inhibiting the action of the ferments present in the inflammatory exudate. An important factor in the acute process under discussion is the immense number of bacilli present. Under these conditions

it is not difficult to imagine a corresponding increase in the amount of inhibiting substances, an increase sufficient to neutralize the ferments liberated by the disintegration of leucocytes and other cells. Caseous areas may persist for a long time without undergoing absorption. The rapid softening of these areas subsequent to secondary infections is probably due not only to the entrance of fresh ferments, but also to the fluid accompanying these conditions, the fluid acting as a diluent and aiding in the removal of the inhibiting substances. Soaps if present must exist in very minute quantities in the bacilli. They are probably formed from the neutral fat of the bacilli. The lipases hydrolyze the fats, and the acids are then saponified. Klotz (12) and Bergell (13) have demonstrated the presence of lipases in tuberculous pus and lymphocytes, while Wells and Corper (14) found the ferment present in the tubercle bacilli.

According to Sata (15), most of the fat present in caseous matter is found at the periphery of the area. This may be due either to a wandering in from the outside, or, more probably, to synthesis from the fatty acids liberated during the disintegration of the cells and of the tubercle bacilli. Our experiments indicate that soaps of the unsaturated fatty acids obtained from tubercle bacilli are the active agents in preventing autolysis, and the identification of these acids will probably aid in determining the origin of the neutral fat found in caseous areas.

In our paper on the ferment-inhibiting substances present in tubercle bacilli (16) we reported that iodin combined with the unsaturated fatty acids obtained from the bacilli and neutralized their ferment-inhibiting properties. We also emphasized the importance of this observation in connection with a specific chemotherapeutic agent for tuberculosis. In our present work we have shown that iodin exerts a similar action on the ferment-inhibiting substances present in caseous matter. Thus the iodin may serve another purpose by aiding in bringing about solution and absorption of the caseous matter, and exposing the bacilli, which otherwise are more or less inaccessible, to the influence of the therapeutic agent.

It is a well known clinical observation that iodides cause tubercle bacilli to appear in the sputum of patients with pulmonary tuberculosis, though they were previously absent. This phenomenon is

probably due to the neutralization of the action of the ferment-inhibiting substances by the iodin. Subsequent to this neutralization, ferment action ensues, with liberation of the bacilli and their appearance in the sputum.

The autolysis that occurs in the center of large anemic infarcts, and the lack of autolysis at the periphery, can be explained in the same manner. At the periphery of the infarct the fatty acids which are liberated following the death of the cells are saponified by the alkalies present in the tissues, and by those coming from the surrounding fluids. The soaps thus formed inhibit proteolysis until the phagocytes, invading the periphery, open up channels through which sufficient fluid can enter to wash out or dilute the soaps so that autolysis can proceed. The phagocytes of course also take part in the removal of the dead tissues. In the center of the infarct the alkalies are soon used up and the tissues then become acid in reaction on account of the excess of fatty acids. Wiener (17) found that autolytic enzymes act best in an acid medium, and this is to be expected as soaps are inactive as inhibiting agents under these conditions.

CONCLUSIONS.

1. Caseous matter obtained from lymph glands which have not become secondarily infected contains substances which inhibit enzyme activity. These substances consist chiefly of soaps of the unsaturated fatty acids.

2. The inhibiting substances are present in relatively smaller amounts when the caseous matter has become secondarily infected. This is probably due to the dilution and washing out of the soaps.

3. Ferments are either entirely absent or present in very small amounts, unless the caseous matter has become secondarily infected.

4. Caseous material from the lungs contains smaller amounts of the inhibiting substances. This may be due to the acuteness of the process, which does not permit an accumulation of the soaps, or to the binding of the soaps with the ferments.

5. Ferments are present in caseous pneumonia. In the whole emulsion the ferments are less active in an alkaline than in an acid reaction; but removal of the soaps shows that those active in an alkaline reaction are also present in considerable amounts.

6. The previous treatment with iodin of caseous matter from both lymph glands and lungs increases the action of the trypsin.

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THE NATURE OF SERUM ANTITRYPSIN.

Studies on Ferment Action. XIII.*

By JAMES W. JOBLING, M.D., and WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The efforts that have been made to determine the nature of the ferment-inhibiting substances of the blood give no definite information concerning their significance or their character. At one time it was thought that the antitryptic index of the serum would prove to be a valuable aid in the diagnosis of cancer.

Brieger and Trebing (1) stated that 90 per cent. of the patients suffering from carcinoma or sarcoma, whom they had examined, showed an increase of antitrypsin in the blood. Von Bergmann and Meyer (2) confirmed this observation, though they also found a similar increase in 24 per cent. of non-cancerous patients. Recent work indicates that the increased ferment-inhibiting action of the serum cannot be relied upon as a diagnostic test for cancer. It is frequently present in the acute infections, such as pneumonia, typhoid fever, etc.; in chronic infections, such as tuberculosis and syphilis; in Graves' disease; and in severe anemias. The action of the serum has been ascribed to chemical constituents and to specific immune bodies which act as antiferments. Meyer (3) believes that the antiferment is a true antibody, and that the ferments of the tissue cells act as antigens. He concludes that trypsin and leucoprotease are not so important in this respect. Eisner (4) and Wiens (5) also believe that the antiferment is a true antibody, but that the antigen is the ferment liberated by the polymorphonuclear cells. On the other hand, Jürgensen (6) found no relation between the antitryptic index and leucocytosis. Halpern (7) inoculated dogs with the pancreas of dogs, and found an increase in antitrypsin, but no increase in antipepsin. Eisner (4) studied the inhibiting action of serum against various ferments, and concluded that it possessed a special affinity for trypsin. Some writers have asserted that the serum is more active against trypsin of the same species, but Weil (8) and others have disproved this.

Morgenroth (9) thought that he obtained a specific antirenin by inoculating animals with renin, while Achalme (10) states that he produced a specific antitrypsin by inoculating animals with trypsin. Other investigators repeated the work of Morgenroth and Achalme and have failed to confirm their results. Hamburger (11) showed that sodium chloride destroys pepsin in a neutral solution, thereby invalidating the evidence that a specific antipepsin is present in the blood; immune bodies are active only in a neutral or slightly alkaline reac-

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tion. We must conclude, then, that sufficient proof has not been advanced to support the view that the ferment-inhibiting substances of the blood are true antibodies.

Hedin (12) has shown that charcoal will bind trypsin and that the binding is proportional to the quantity of charcoal and to the time of interaction. Strong acids and alkalies quickly destroy trypsin, but Chittenden and Cummins (13) found it to be much more resistant to these agents in the presence of protein. Kudo (14) states that all the mineral acids are active in this respect, sulphuric acid being active in a dilution of one in a thousand, while the organic acids are much less so. Bayliss (15) and Abderhalden and Gigon (16) found that some of the products of tryptic activity possess the property of inhibiting the action of the ferment, the free amino acids being more active than the polypeptids.

Schwartz (17) concluded that the antitryptic activity of the serum is due to the lipoids. He found that he could partially inactivate sera by extracting them with ether, and that the sera could be reactivated by the addition of lecithin. Sugimoto (18) observed a decrease in the antitryptic strength of sera after extraction with ether and benzol, and concluded that the lipoids were the active constituents. He believes that a complete extraction of the fat, and the dissociation of the lipoid-protein combinations will cause a removal of the ferment-inhibiting action. He also tested alcoholic extracts which were prepared from egg-white, beef brain, and beef liver, and found them inactive as anti-enzymes when used as emulsions in solutions of sodium chloride. Meyer (3) suggests that the effect of the ether was due to the destruction of the anti-enzymes and not to their extraction. He also refers to the normal antitryptic index found in the lipoid-rich sera of luetics and diabetics. He found that it was not destroyed by extracting the dried serum with ether, and the fluid serum with petroleum ether, but states that this does not disprove the possibility of its being a lipoid-protein combination. He does not believe that protein cleavage products are the inhibiting agents. Cobliner (19) assumes that lipoids are not the active agents, as dried sera extracted with ether, chloroform, or petroleum ether do not lose their antitryptic activity. He found that the inhibiting action was increased if the serum was shaken with olive oil. Kirchheim (20) concludes that the antiferment merely prolongs the action of the trypsin and does not destroy it. He observed that chloroform reduced the inhibiting action of the serum.

Rosenthal (21) believes that the action of the serum is due to protein cleavage products, and that its increase in carcinoma and other diseases with cachexia can be thus explained.

Two years ago we observed that sera preserved with chloroform soon lost their antitryptic action. The same observation had been made previously by Delezenne and Pozerski (22), but we did not know of it. This discovery convinced us that the ferment-inhibiting substances of the serum were lipoids, and that they were soluble in fat solvents. We (23) have published the results of some preliminary work based on this idea, in which it was shown that the soaps of the unsaturated fatty acids obtained from various sources possessed the property of acting as anti-enzymes. These results caused us to study the ferment-inhibiting action of the blood to see if it was due to the same agents.

The Fuld-Gross technique was used by most of the investigators engaged in the study of the anti-enzymes of the blood. We have already discussed in

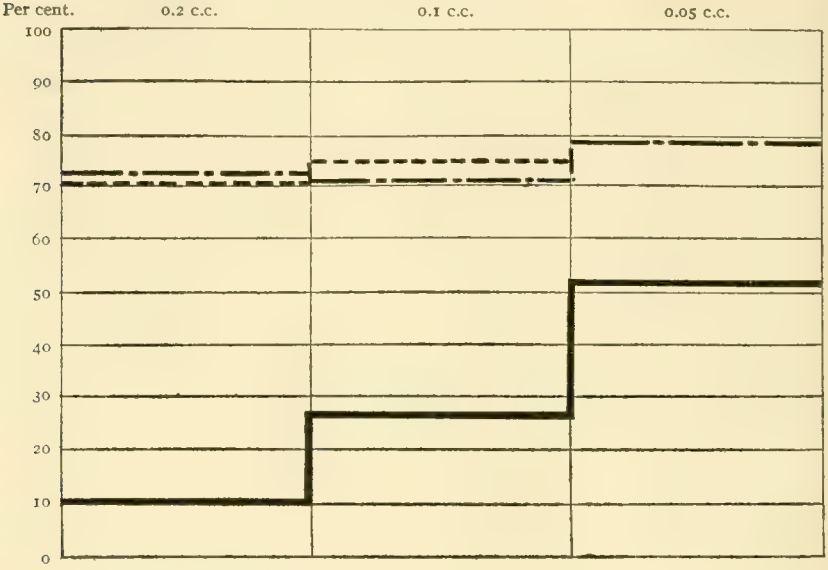
another paper (*23*) the difficulties we encountered in the use of this method. In this work, as well as in that previously reported, our interpretations were based upon the total incoagulable nitrogen determined according to the method recommended by Folin.

INFLUENCE OF CHLOROFORM OR ETHER EXTRACTION ON SERUM ANTITRYPSIN.

Considerable work has been done with lipoid solvents in order to show the relation which exists between the lipoids of the serum and antitrypsin, but with contradictory results. In most of the work the solvent was allowed to act for a short time only, and, in some instances, on the dried serum. In the first experiment we wished to determine the influence of chloroform or ether on the enzyme-inhibiting action of the serum. Fresh dog serum was diluted 1 to 5 with salt solution, and divided into three parts. To one part was added chloroform; to another ether; the remaining portion was preserved without the addition of any agent. The three flasks containing the sera were then kept at room temperature for forty-eight hours. The mixtures were shaken frequently during this time. Before testing, the excess of chloroform or ether was removed with pipettes, and the serum then filtered through filter paper until clear. They were mixed in various dilutions with trypsin and placed in the incubator for thirty minutes. Casein was then added and the mixture incubated for two hours at 37° C. A control flask containing only trypsin and casein was incubated for the same period of time. The lines on text-figure 1 show the amount of digestion in each flask as compared with that obtained in the control tube which contained no serum. This method will be followed in all the subsequent experiments.

Text-figure 1 shows the results of extracting the serum with ether or chloroform. The lines in the text-figure show that in the tube containing 0.2 of a cubic centimeter of the unextracted serum, the digestion was only 10 per cent. of that obtained in the control, while in the tubes containing the extracted serum, the digestion was 70 per cent. of the control. In other words, the chloroform and ether had removed 60 per cent. of the inhibiting action of the serum. Dog serum has not the high anti-enzyme power of human serum, and this accounts for the comparatively large amount

used. This experiment has been repeated many times with human serum, horse serum, dog serum, and guinea pig serum, and similar results have been obtained with each. It was found necessary to shake the mixtures of chloroform and ether frequently, otherwise the inhibiting action of the serum was not removed. At least twice as much solvent as serum should be used.



Black line = original serum.
Dotted line = ether-extracted serum.
Broken line = chloroform-extracted serum.

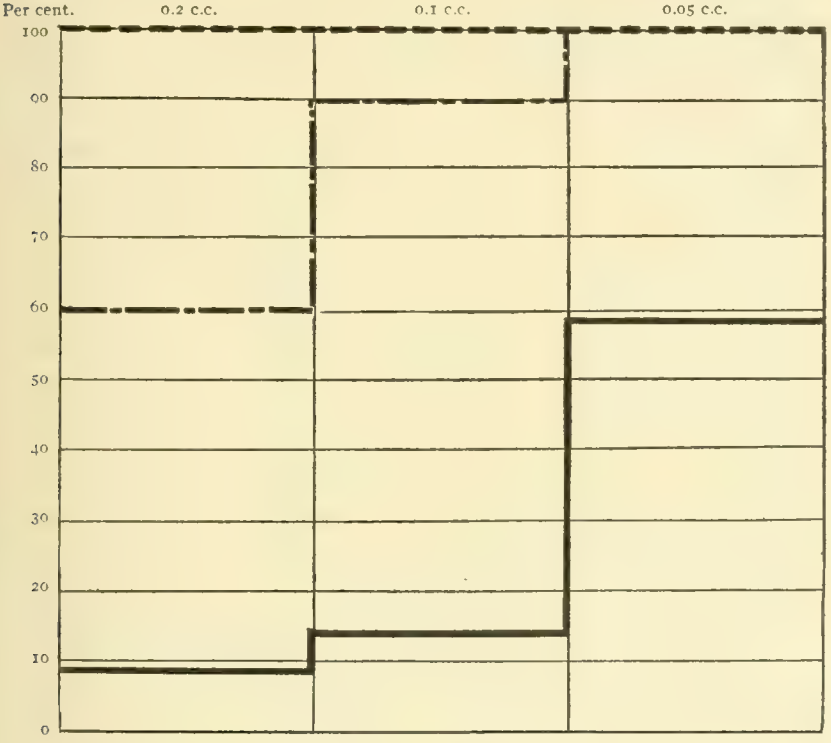
TEXT-FIG. 1. Effect of ether or chloroform extraction on serum antitrypsin.

Two possibilities must be considered in explaining the action of chloroform or ether on the enzyme-inhibiting action of the serum: first, that the substances causing the inhibiting action have been destroyed; and, second, that they have been taken up by the solvents.

INFLUENCE OF THE REACTION ON EXTRACTED SERA.

The next experiment was conducted to see if the activity of the serum could be restored by rendering it neutral or slightly alkaline in reaction. In order to duplicate the conditions present in the last experiment, ether was added to the serum in two tubes, and the

mixtures were kept at room temperature. A third tube of serum was kept without any preservative. After four days the ether was evaporated from one tube, the serum shaken thoroughly, and then made slightly alkaline with a dilute solution of sodium hydrate. This was done to see if the acids could be saponified, and the activity



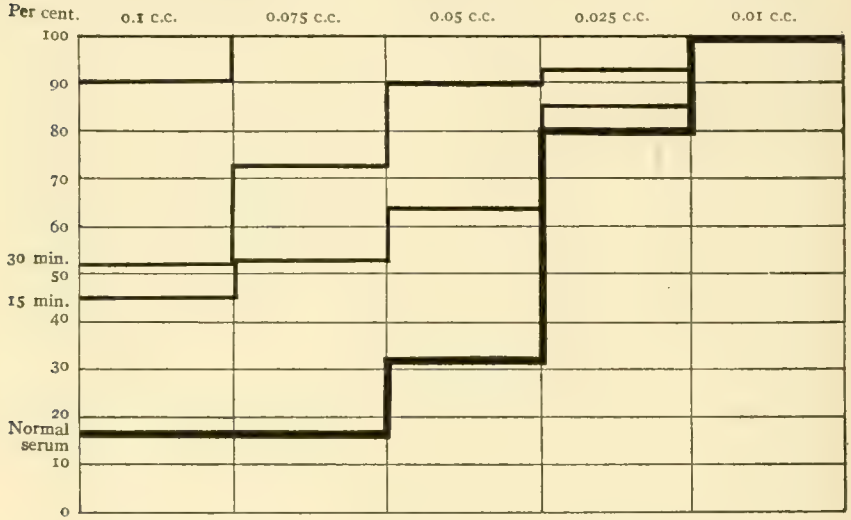
Black line = human serum.
Dotted line = ether-extracted human serum.
Broken line = ether-extracted human serum, ether evaporated.

TEXT-FIG. 2. Effect of evaporating ether extract and partial reactivation of serum.

of the serum restored. The ether was removed from the second tube by means of a pipette, and the serum was then filtered several times through filter paper. It was made neutral by adding a dilute solution of sodium hydrate. Text-figure 2 shows the results obtained in this experiment. The chart shows that only 8 per cent.

of digestion was obtained in the flask containing 0.2 of a cubic centimeter of the untreated serum, while 100 per cent. of digestion was obtained in the flask containing the same amount of ether-extracted serum. The serum which had been made slightly alkaline after evaporation of the ether showed about half the inhibiting action possessed by the one to which nothing had been added. These results indicate that the solvent does not destroy the anti-enzyme.

We must assume that the action of the alkali is due to a combination with some substance soluble in ether, as the alkali did not re-



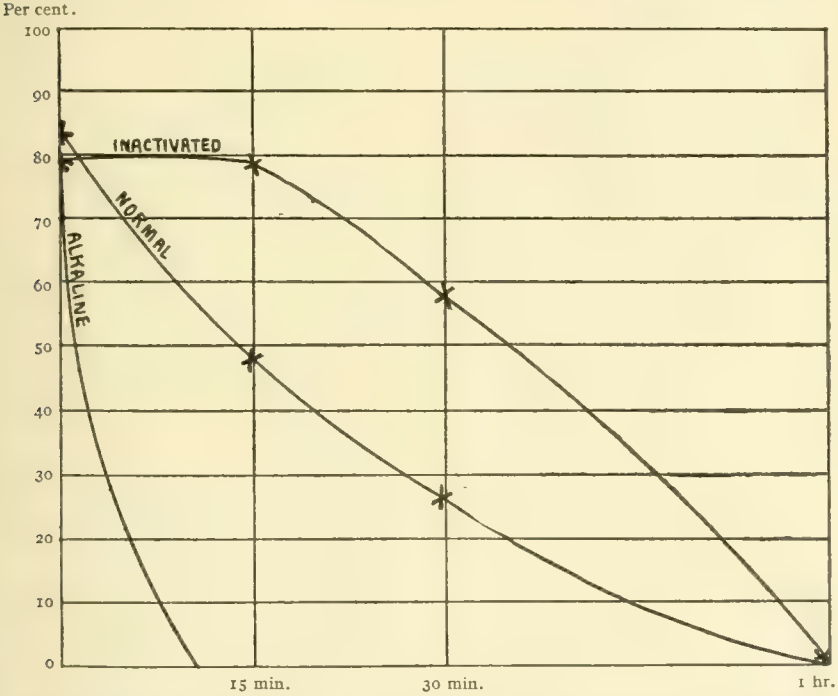
TEXT-FIG. 3. Effect of chloroform on serum antitrypsin at 37° C.

store the activity of the serum from which the ether had been removed, but partially restored it to the serum from which the ether was evaporated. Under these conditions the alkali would not be likely to combine with lipoids such as lecithin, cholesterol, etc., and so only the fatty acids remain. It cannot be expected that the full strength of the inhibiting action observed in the untreated serum should be obtained here. Following the evaporation of the ether, the extractives would be more or less insoluble in the serum and this would render it impossible to saponify all the free fatty acids without the aid of heat and an excess of alkali. If the removal of the inhibiting action at room temperature by chloroform is aided by the action of the serum lipase, a shorter time should be required

by incubating the serum at 37° C., ferments being more active at this temperature.

TIME REQUIRED FOR CHLOROFORM EXTRACTION AT 37° C.

In the next experiment we wished to determine the length of time required for chloroform to remove the ferment-inhibiting action of the serum when the mixture was kept at 37° C. Chloroform was added to fresh serum, thoroughly shaken, and the



TEXT-FIG. 4. Rate of removal of serum antitrypsin in normal, inactivated, and alkalinized serum at 37° C.

mixture was placed in the incubator at 37° C. At fifteen minute intervals some of the serum was removed and tested for its ferment-inhibiting properties. In separating the serum from the chloroform it is necessary to centrifuge the emulsion at high speed for about five or ten minutes, and then to filter it through coarse filter paper several times until the serum is quite clear and all chloroform evapo-

rated. This experiment demonstrates that the ferment-inhibiting action of the serum is almost wholly lost after sixty minutes' incubation with chloroform (text-figure 3).

In the next experiment we wished to determine if lipase was essential to the removal of the inhibiting substances by the chloroform. The serum was divided into three portions. One portion was heated at 54° C. for thirty minutes in order to destroy the lipase; the second portion was made distinctly alkaline in order to prevent the serum from becoming acid; and the third portion was used as a control. Chloroform was added to all three portions and they were then placed in the incubator. A portion of each lot was removed at fifteen minute intervals and tested against trypsin for its anti-enzyme properties (text-figure 4).

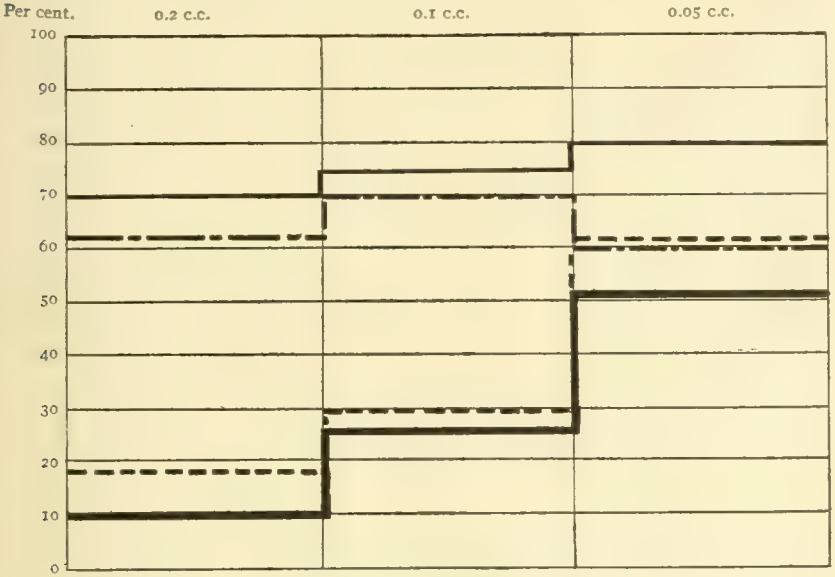
The results show that lipase is not essential to the removal of the inhibiting substances by the chloroform, and indicate that the inhibiting substances are probably fatty acid esters which are soluble in chloroform and ether. There was very little decrease in the inhibiting action of the inactivated serum during the first fifteen minutes of incubation. This may have been due to the absence of the lipase, or to some physical change in the serum caused by heat.

FATTY ACIDS AS ANTIFERMENTS.

The results obtained in the previous experiments indicate that the inhibiting agents are compounds of the fatty acids, and the next experiment was planned to see if this was the case.

Ether was added to fresh dog serum and the mixture was allowed to stand for two days at room temperature. The ether was then removed and divided into two portions. One portion was made slightly alkaline with sodium alcoholate and evaporated to dryness. The soaps were then dissolved in water and filtered; the acids liberated with hydrochloric acid, taken up in ether, and resaponified. The second portion of ether was shaken with water and the ether then evaporated at low temperatures. The soaps prepared from the first portion, the emulsion prepared from the second, and the serum from which the ether extracts were obtained, were tested for their ferment-inhibiting action. The original untreated serum was used

as a control. Text-figure 5 shows the percentage of casein digested by trypsin after it had been incubated for thirty minutes with these preparations. The lines on the chart show that the soaps prepared from the ether extract cause nearly as much inhibition as the untreated serum, while the water emulsion gives 62 per cent. of diges-



Heavy black line = original serum.
Lighter black line = extracted serum.
Dotted line = ether extract saponified.
Broken line = ether extract emulsified.

TEXT-FIG. 5. The antitryptic effect of the ether extract of normal serum when saponified.

tion. The inhibition caused by the emulsion is probably due to some of the acids combining with the alkali which had been used in the preparation of the casein solution. The soaps thus formed would of course inhibit ferment action. The original untreated serum gave 10 per cent. of digestion, and that extracted with ether 70 per cent. Complete removal of the inhibiting action of the serum is rarely obtained in less than four days' extraction at room temperature, and then, as a rule, only with chloroform.

Up to the present time all observers have failed to secure any

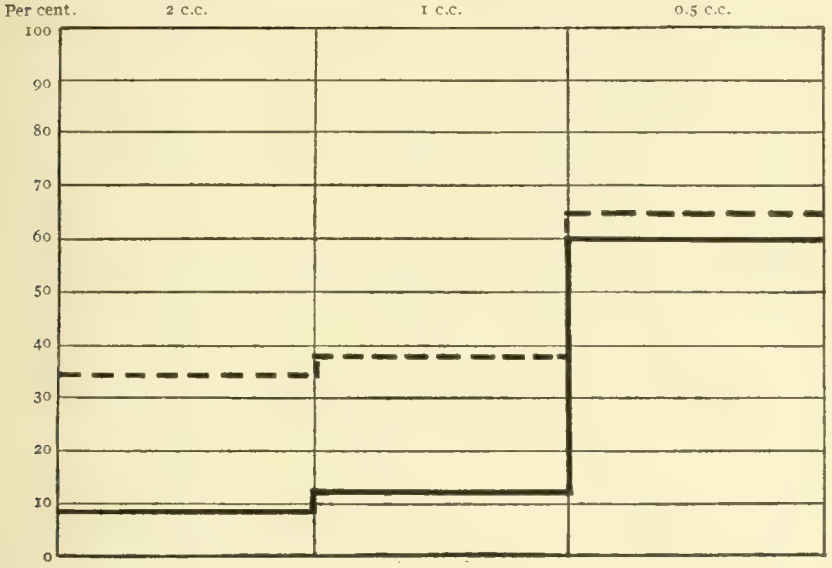
antitryptic effect of the ether or chloroform extracts. This is due solely to the fact that in the simple emulsions with which they worked, the intimate contact of the trypsin and the unsaturated fatty acid complex, which is the basis of the antitryptic activity, is not brought about. In the native serum these lipoids are in a state of dispersion sufficiently great to permit such contact, and we can reproduce it only by saponifying these fatty acid radicals and thus bringing them into a colloidal solution again. We have found that we can at all times secure the antitryptic effect in almost its original value from the extracts, if care is taken to secure complete saponification. If such extracts are not well saponified, so that droplets of unsaponified material can be observed under the high power microscope, then either little or none of the antitryptic effect will be recovered. The results of this experiment indicate that the inhibiting action of the serum is due to the fatty acids. It is not necessary to assume that they can act only as soaps; probably the esters containing unsaturated fatty acids are just as effective.

REMOVAL OF ANTIFERMENT BY FILTERING ACID SERUM THROUGH KAOLIN.

The results that we have been discussing were probably due to the extraction of the fatty acids by the solvent. The fatty acids can also be liberated by means of other acids, in which case they should be thrown out of solution and removed by filtration. The next experiment was planned to find out if the inhibiting substances could be removed by filtering through kaolin serum which had been made strongly acid with hydrochloric acid.

Twenty cubic centimeters of fresh dog serum were diluted with forty cubic centimeters of water, and five cubic centimeters of N/10 hydrochloric acid were added. The mixture was shaken thoroughly, allowed to stand for a few minutes, and then filtered several times through kaolin. After standing for a short time to permit all the serum to drain off, the kaolin was heated with sodium alcoholate. The mixture was then filtered and the filtrate evaporated to dryness. The soaps were dissolved in water and tested for their ferment-inhibiting properties. The acid serum obtained after filtration was made neutral and also tested. Some of the original unacidified

serum was filtered through kaolin to see if kaolin filtration would exert any influence on normal serum. Text-figure 6 shows the results of filtering acidified serum through kaolin. The inhibiting action of the serum which was made acid and then filtered through kaolin was completely lost, and is therefore not shown in the chart. The soaps prepared from the kaolin were active as inhibiting agents

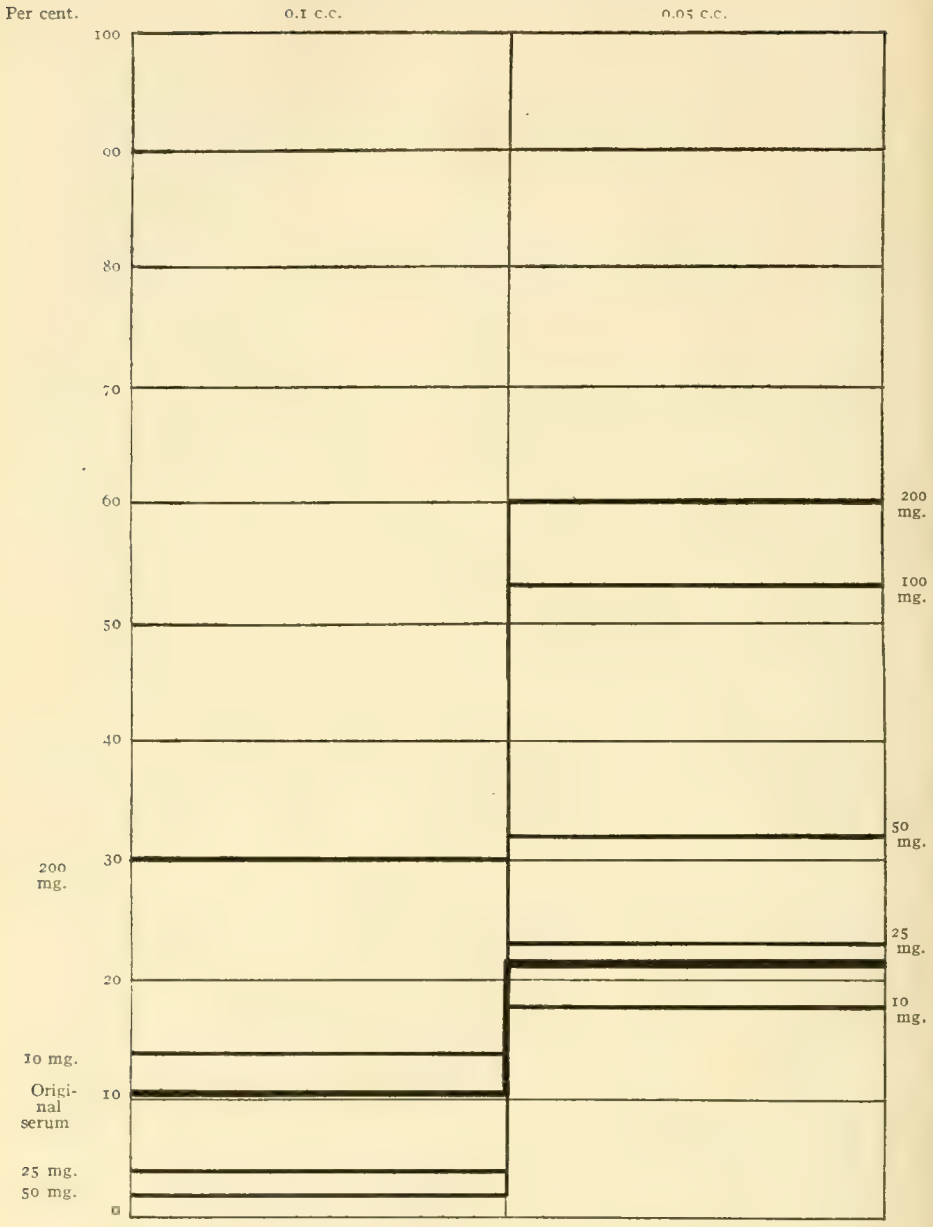


Black line = serum diluted by 10 (horse).
Dotted line = kaolin extract saponified, *i. e.*, $\frac{1}{10}$ strength of serum.
TEXT-FIG. 6. Antitryptic effect of kaolin extract saponified.

and indicate that the fatty acids are the active substances present in the serum. The strength of the soap solution was about 0.1 of that of the untreated serum. We have not been able by this method to recover a larger amount of the inhibiting substances, though the serum has usually lost all its anti-enzyme properties.

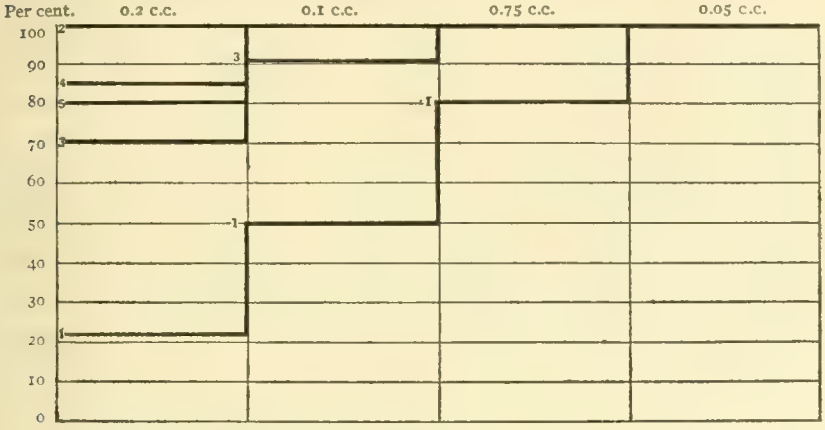
INFLUENCE OF IODIN OR POTASSIUM IODIDE ON ANTIFERMENT.

In our previous work (23) we have shown that soaps of the unsaturated fatty acids lose their ferment-inhibiting properties when treated with iodin. For this purpose we used iodin dissolved in a solution of potassium iodide and removed the excess of iodin by



TEXT-FIG. 7. Effect of varying amounts of potassium iodide on serum antitrypsin.

shaking the mixture with chloroform. This last step was necessary, as iodin inhibits tryptic activity. In the present work we were unable to use the iodin-iodide of potassium mixture, as we have shown that serum loses some of its ferment-inhibiting properties even when shaken with chloroform for a short time. For these reasons we have first investigated the action of potassium iodide alone on the ferment-inhibiting action of the serum.

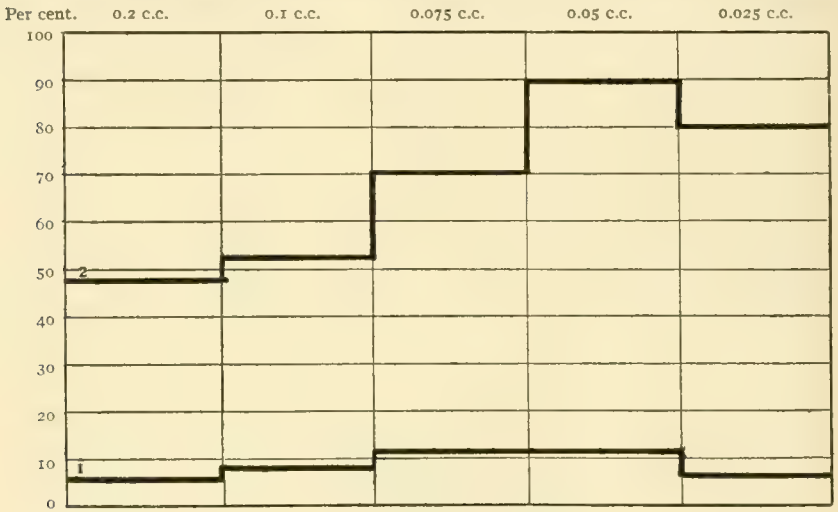


1 = dog serum, 3 days at room temperature.
2 = 1 c.c. dog serum + 200 mg. potassium iodide, 3 days at room temperature.
3 = 1 c.c. dog serum + 100 mg. potassium iodide, 3 days at room temperature.
4 = 1 c.c. dog serum + 200 mg. sodium iodide, 3 days at room temperature.
5 = 1 c.c. dog serum + 100 mg. sodium iodide, 3 days at room temperature.

TEXT-FIG. 8. Effect of sodium iodide and potassium iodide on antitrypsin *in vitro*.

Fresh human serum was divided into six portions of five cubic centimeters each. To one of these portions, potassium iodide was added in the proportion of 10 milligrams to one cubic centimeter; to the second, 25 milligrams to one cubic centimeter; and to the third, 50 milligrams; to the fourth, 100 milligrams; and to the fifth, 200 milligrams. The sixth received no potassium iodide and was used as a control. The tubes containing the sera were placed in the incubator for three hours and afterwards kept at room temperature over night. The following morning they were incubated for thirty minutes with trypsin, and the casein was then added. Control tubes containing trypsin and potassium iodide, without serum, showed

that the chemical did not inhibit the activity of the trypsin. Text-figure 7 shows the influence of potassium iodide on the ferment-inhibiting action of the blood. The chart shows that potassium iodide even under these conditions destroys some of the ferment-inhibiting action of the serum. In the first column in which 0.1 of a cubic centimeter was used, the influence was not marked; but in the second column in which 0.05 of a cubic centimeter of serum was used, there was considerable diminution of the inhibiting action.



1 = guinea pig serum (1 c.c. diluted by 10), normal.
2 = guinea pig serum (1 c.c. + 200 mg. potassium iodide), over night in incubator.

TEXT-FIG. 9. Effect of potassium iodide on antitrypsin of guinea pig serum.

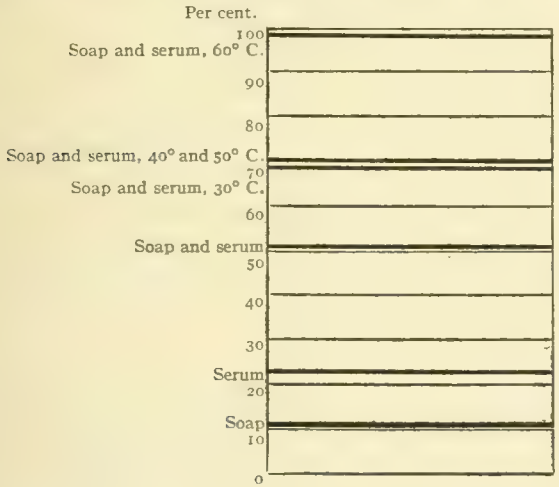
Another experiment was made to see if other iodides would act in a similar manner. In this instance fresh dog serum was treated with sodium iodide and potassium iodide. The technique used was the same as that described for the preceding experiment except that the mixtures were kept for two days at room temperature. The results of this experiment (text-figure 8) are more striking than those of the preceding one. This may be due to the fact that the iodides were in contact with the serum twenty-four hours longer. The sodium iodide is just as active as the potassium iodide in removing the inhibiting action of the serum.

Still more conclusive are the results obtained with guinea pig serum, as this serum is more active as an inhibiting agent. The mixture of serum and potassium iodide stood in the incubator over night. Text-figure 9 shows the influence of potassium iodide on guinea pig serum.

The effect of potassium iodide on the serum antitrypsin seems to be most marked on fresh sera. Treatment of serum with an equal volume of strong solution of hydrogen peroxide, and incubated over night, also causes complete loss of its antitryptic power.

INFLUENCE OF HEAT.

Serum loses its ferment-inhibiting properties if it is heated for thirty minutes at 65° C. If the fatty acids are the inhibiting agents, they must undergo some physical or chemical change which renders them inactive. In the next experiment we wished to determine the



TEXT-FIG. 10. Effect of heat on the antitryptic activity of soap and serum. 1 c.c. contains 0.1 c.c. of serum and 0.01 gm. of soap.

influence of heat on the ferment-inhibiting properties of soap when it is mixed with serum. We had found that soaps dissolved in water did not lose their activity when heated at 100° C., and so it was of interest to determine if they were more susceptible in the presence of serum protein.

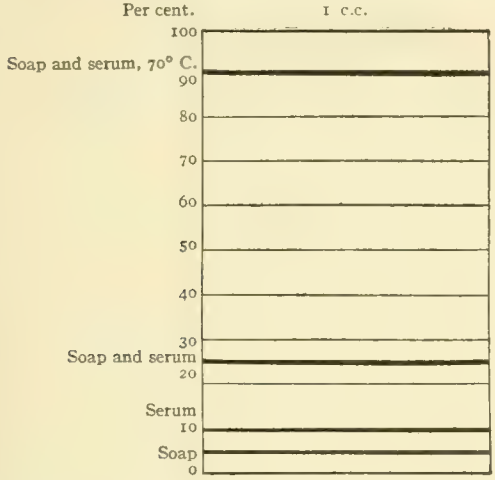
Linseed oil soap, with an iodin value of 110, was used in this experiment. A 10 per cent. solution of dog serum was prepared and to this was added an equal amount of a 1 per cent. soap solution. Portions of the mixture were heated for thirty minutes at 30°, 40°, 50°, and 60° C., and then tested for their ferment-inhibiting properties. The unheated mixture of soap and serum and the unheated soap and serum solutions were tested separately as controls (text-figure 10). The chart shows that the soap solution was active as it permitted only 12 per cent. of digestion, while the serum was less active, permitting 23 per cent. of digestion. The unheated mixture of soap and serum gave 52 per cent. of digestion, while the mixtures heated at 30°, 40°, and 50° C. gave 70 per cent. In the mixture heated at 60° C. the inhibition of ferment action was completely lost.

The amount of inhibition obtained with the unheated mixture of soap and serum was less than that obtained with the soap or the serum alone. This indicated that some of the inhibiting action was destroyed by mixing the serum and soap. In our previous work we found that a slight degree of acidity was sufficient to destroy the activity of the soaps and so we immediately tested the serum used in this experiment. It was found that one cubic centimeter of the serum required 0.1 of a cubic centimeter of an N/10 sodium hydrate solution to neutralize it. This explains why the mixture of soap and serum was less active than the soap or serum separately. The high degree of acidity was probably due to the fact that the serum was several weeks old.

Experiments were then conducted to determine the influence of heat on soap mixed with neutral serum. The same acid serum was made slightly alkaline to phenolphthalein by means of a sodium hydrate solution. Text-figure 11 shows the influence of heat on linseed oil soap when mixed with neutral serum.

Here again the unheated mixture of soap and serum does not inhibit the action of trypsin as much as the soap or the serum alone, though it does show more inhibition than when the serum was acid in reaction. It is possible that here, also, there was some dissociation of the soaps which caused a decrease of inhibiting action. Heating at 70° C. caused almost complete loss of activity of the soap-serum mixture.

Further experiments were made with serum which had been rendered distinctly alkaline by adding 0.1 of a cubic centimeter of N/10 sodium hydrate solution to the neutral serum. This alkaline serum-soap mixture lost only 30 per cent. of its inhibiting proper-



TEXT-FIG. 11. Effect of heat on the antitryptic activity of soap and serum in neutral reaction.

ties when heated for thirty minutes at 70° C. In other words, loss of activity as the result of heating appears to bear some relation to the reaction of the serum.

DISCUSSION.

The results obtained in the preceding experiments justify, we think, the conclusion that the ferment-inhibiting properties of the serum are due to the presence of compounds of the unsaturated fatty acids. Their solubility in ether or chloroform suggests that they are not in the form of soaps, but are present as esters. In some experiments which are not described in this paper, we found that a fine emulsion of olein in serum inactivated by heat, or in salt solution, possessed some ferment-inhibiting action, the inhibiting action bearing a distinct relation to the degree of dispersion. This observation is significant as numerous investigators have reported that the antitrypsin of the serum is increased after eating.

In our study of the ferment-inhibiting substances present in tuberculous caseous material (24) we at first thought that the unsaturated fatty acids were present in the form of soaps, but subsequent experiments showed that they could also be extracted by means of chloroform or ether, after which the material was digestible by trypsin. These results indicate that here also the acids in the form of esters are active as inhibiting agents.

If fresh serum is tested daily for its ferment-inhibiting action it will be found that its strength decreases from 5 to 10 per cent. for the first few days; after that it remains almost constant for an indefinite period of time. With this decrease in anti-enzyme activity there is an increase in acidity. The acidity is probably due to the liberation of fatty acids by the action of the serum lipase on the fatty acid compounds present. If serum lipase is mixed with ethylbutyrate and then incubated, the degree of acidity will be found to be practically the same at the end of four hours as after twenty-four hours. But if the mixture is made neutral at the end of the first four hours and again incubated, the acidity at the end of the second four hours will be almost equal to that obtained after the first incubation. In other words, the ferment can act to a certain point only; after that its action is inhibited either by the amount of acids liberated or by the synthetic compounds formed from the acids. When chloroform or ether is added to the serum, the acids are taken up by the solvent almost as fast as they are liberated, and so they do not interfere with the action of the lipase. Under these conditions the ferment remains active until most of the substrate consisting of the fatty acid compounds has been destroyed. We have found that chloroform acts better than ether in removing the inhibiting agent, and this is to be expected as chloroform is known to exert less influence on ferment activity. This hypothesis also explains why a serum can be left for months with other preservatives without losing all its antiferment properties. In order to remove the inhibiting substances with chloroform it is necessary that a large amount of the solvent be used, and that the mixture be shaken frequently.

Two possibilities must be considered in explaining the destruction of the ferment-inhibiting properties of the serum by heat: first,

it might be due to saturation of the unsaturated fatty acids; second, to the mechanical inclusion of the fatty acid compounds in the fine coagula caused by the heating. If the fatty acids become saturated when heated with protein the combination formed must be easily dissociated, as their activity is not permanently lost. If an inactive soap-serum mixture is saponified with strong solutions of sodium hydrate, and the fatty acids are then liberated in the usual manner and resaponified, it will be found that the soaps are just as active as those obtained from an unheated portion of the same mixture. At first we thought the destruction of the inhibiting action was due to the mechanical inclusion of the fatty acid compounds in the fine coagula caused by heating, but subsequent experiments with sera made strongly alkaline with sodium hydrate disproved this. Under these conditions, coagula would not form, yet the resistance of the inhibiting substances to heat was greatly increased. We have noticed frequently that there is an increase of from 5 to 20 per cent. in the antiferment activity of sera heated for fifteen minutes at 54° to 55° C., especially in sera that are lipemic.

Thiele (25) found that the neutral fat of chyle was adsorbed by serum when the mixture was kept for several hours at 37° C. The fat in such a mixture could not be extracted with ether, but the combination could be broken up by peptic digestion and by treatment with alcohol. A similar explanation may apply to our observation that a soap-serum mixture heated at 70° C. loses its anti-enzyme properties. The iodin values of the heated and unheated mixtures are the same. We must therefore assume that the combination formed is one which is readily dissociated.

The effect of the potassium iodide is probably due to the liberation of small amounts of free iodin which can then saturate the unsaturated carbon bonds. Thus the iodin value (determined by precipitating the serum proteins by alcohol and titrating the alcoholic filtrate) of a serum treated with potassium iodide falls to almost one fifth of the original value. Free iodin dissolved with a small amount of potassium iodide also lowers the antitryptic activity. The relation of the iodide to serum antitrypsin will be discussed in a later paper. The influence of iodin in removing the inhibiting action of the serum shows that the inhibiting agents belong to the unsaturated group of the fatty acids.

The effect of hydrogen peroxide in completely destroying the antitryptic activity; the fact that the extracted lipoids, which are the active agents, are all unsaturated compounds, having an iodin value of from fifty to sixty; and also the fact that sera with a high antitryptic index give a higher iodin value than sera with a low antitryptic index, all tend to confirm this view from a chemical standpoint. From the clinical investigations so far reported there is similar confirmation; there is an increase of the antiferment after eating; in the acute infections such as typhoid fever, pneumonia, etc.; in chronic infections such as syphilis and tuberculosis; in Graves' disease; and in the severe anemias, such as those accompanying malignant growths, etc. In all but the first of these conditions, the demands of the body or the action of the toxins, cause a marked inroad into the fat depots of the body. This disappearance of fat in conjunction with a lowering of the oxidation powers of the liver and tissues in general causes an increase in the amount of unsaturated fatty acids in the blood and thus an increase in the ferment-inhibiting action.

CONCLUSIONS.

1. The ferment-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids.

2. These fatty acid compounds may be removed from the serum by means of chloroform or ether.

3. Soaps prepared by saponifying the chloroform or ether extracts inhibit the action of trypsin.

4. The anti-enzyme action of the serum can be removed by filtering acid serum through kaolin, and can in part be restored by extracting the kaolin.

5. The decrease in strength of anti-enzyme in old sera is probably due to the action of the serum lipase.

6. Iodin, potassium iodide, or hydrogen peroxide remove the inhibiting action of the serum.

7. Soaps of the unsaturated fatty acids lose their ferment-inhibiting action when heated with serum at 70° C.

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SEROTOXIN.

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SEROTOXIN.

STUDIES ON FERMENT ACTION. XIV.*

BY JAMES W. JOBLING, M.D., AND WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

In a previous paper (1) we have demonstrated the lipoidal nature of serum antitrypsin and have discussed the various methods which may be employed to remove the antitrypsin from the serum; *i. e.*, by extraction, or by the saturation or oxidation of the unsaturated carbon bonds of the fatty acids on which the antitryptic property depends. In view of the fact that practically all sera contain proteolytic ferments, we undertook to determine whether or not sera from which the protective substance had been removed, with resulting exposure of the serum proteins, would be toxic for the homologous animal; and if so whether complete removal of the lipoids was necessary to produce toxic effects. Apart from the interest that such a study would have in relation to the production of the so called anaphylatoxins, we felt that numerous pathological conditions, which at present are not wholly understood, might possibly have their basis in a protein intoxication,—a true auto-intoxication in the sense that the toxic substances were formed from the serum or cellular protein of the host, and without reference to the gastro-intestinal tract. We have in mind particularly such conditions as arteriosclerosis, nephritis, asthma, and acute acidosis in infants. The work of Longcope (2) supports this idea. He observed that repeated injections of egg albumen into sensitized dogs and cats caused nephritis and other organic lesions.

TOXICITY OF SERA WHICH HAVE BEEN RENDERED ACID AND THEN FILTERED.

Our first experiments were made with serum from which the lipoids had been removed by acidifying the serum and then filter-

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ing it through hard paper filters and kaolin, or through a Berkefeld filter. We have shown that part of the antitrypsin can be recovered from such filters by saponification.

0.2 of a cubic centimeter of N/10 sulphuric acid was added to each cubic centimeter of fresh guinea pig serum, and the mixture was filtered several times through a hard paper filter in the evening. It was then placed on ice over night, and on the following morning it was neutralized before injection. In all instances unless otherwise stated the injections were given intravenously.

Amount injected.	Weight of guinea pig.	Dose per gm. of weight.
4 c.c.	250 gm.	0.016 c.c.

This dose given intravenously caused the immediate symptom complex of anaphylaxis with death of the animal in two minutes. The autopsy findings were typical,—lungs emphysematous, heart beating, and blood fluid.

In the next experiment 4 c.c. of N/4 hydrochloric acid were added to 20 c.c. of guinea pig serum and left over night in the ice box. Before injection it was passed through a Berkefeld filter.

Amount injected.	Weight of guinea pig.	Dose per gm. of weight.
5 c.c. (not neutralized).	330 gm.	0.015 c.c.

There were marked respiratory convulsions, defecation, scratching of the nose, and the animal was weak for several hours, though it finally recovered.

Amount injected.	Weight of guinea pig.	Dose per gm. of weight.
5 c.c. (neutralized).	250 gm.	0.02 c.c.

Typical death in three minutes. At autopsy the lungs were emphysematous, the heart was beating, and there were no intravascular clots.

In both of these experiments the serum from which the lipoids had been removed—not merely rendered inactive by acidifying—had become toxic for its own species in doses from 0.016 to 0.02 of a cubic centimeter per gram of weight. The simple acidification of the serum, which inactivates its antitryptic effect, does not seem to make the sera toxic in the same degree, probably because on reinjection the lipoids are again brought into a more active condition. Some toxic symptoms are, however, elicited by such sera in large doses (five cubic centimeters).

TOXICITY OF SERA AFTER EXTRACTION WITH ETHER OR CHLOROFORM.

We have shown that serum antitrypsin can be removed by ether extraction, and that the antitrypsin can be recovered in an almost quantitative relation from the ether extract by saponification of the extracted lipoids. That such sera are toxic is shown in the following experiments.

Guinea pig serum was placed under ether on Jan. 6 at room temperature and was left for two days. The flask was shaken occasionally.

Amount injected.	Weight of guinea pig.	Dose per gm. of weight.
4 c.c.	330 gm.	0.012 c.c.

Death was immediate. The lungs were emphysematous, the heart had ceased beating, there was a firm clot in the right ventricle, and the gall bladder was distended.

A second preparation of serum was placed under ether on January 8 and permitted to stand at room temperature until January 13. The effect on intravenous injection was as follows:

Dose in c.c.	Weight of guinea pig.	Dose per gm. of weight.	Result.
4 c.c.	330 gm.	0.012 c.c.	Immediate death. Lungs emphysematous. Heart filled with a large clot.
2 c.c.	245 gm.	0.008 c.c.	Immediate death. Findings as above.
1 c.c.	320 gm.	0.003 c.c.	Some respiratory spasms. Looked sick. Complete recovery.

In like manner chloroform will remove the serum antitrypsin, the result being even more rapid than with ether. It is best to mix about two volumes of pure chloroform with one volume of serum and to shake the mixture thoroughly, so that a thick emulsion is formed. After incubation or standing at room temperature the emulsion is centrifuged at high speed, and the supernatant serum is pipetted off and filtered through a coarse filter paper until the serum is quite clear and all the chloroform has evaporated. In the next experiment the serum was extracted with chloroform for two days at room temperature and the serum then removed.

Amount injected.	Weight of guinea pig.	Dose per gm. of weight.
3.5 c.c.	185 gm.	0.018 c.c.

Death was almost instantaneous. The lungs were emphysematous, the heart beating, and the blood fluid.

A similar preparation was kept at room temperature for five days and then tested.

Dose.	Weight of guinea pig.	Dose per gm. of weight.	Result.
4.0 c.c.	280 gm.	0.014 c.c.	Immediate death.
2.0 c.c.	310 gm.	0.006 c.c.	Immediate death.
1.0 c.c.	320 gm.	0.003 c.c.	Immediate death.
0.5 c.c.	365 gm.	0.0013 c.c.	Respiratory spasm. Looked sick. Complete recovery.

TOXICITY OF IODIZED SERA.

We have shown that the antitryptic effect of the serum can be completely destroyed by agents that oxidize or saturate the unsaturated bonds of the fatty acids, as for instance with potassium or sodium iodide, free iodin, or hydrogen peroxide. The iodide can not be used for the preparation of serotoxin because to lower the antitryptic index relatively large amounts must be used and these salts in themselves injected intravenously in small doses will produce toxic effects similar to the toxic effects of the serum.

Free iodin dissolved in serum containing only a trace of potassium iodide can be readily used for our purpose, according to the method described by von Dungern and Hirschfeld (3). With an excess of iodin a heavy precipitate is thrown out of the serum, but this is redissolved on the addition of an excess of sodium hydroxide, and the serum can then be brought back to a neutral reaction and will remain clear. It requires about two days at incubator temperature to remove completely the antitryptic action. Such sera remain sterile and will not autolyze if an excess of iodin is added. The results of an intravenous injection of an iodized serum which no longer had any antitryptic effect were as follows:

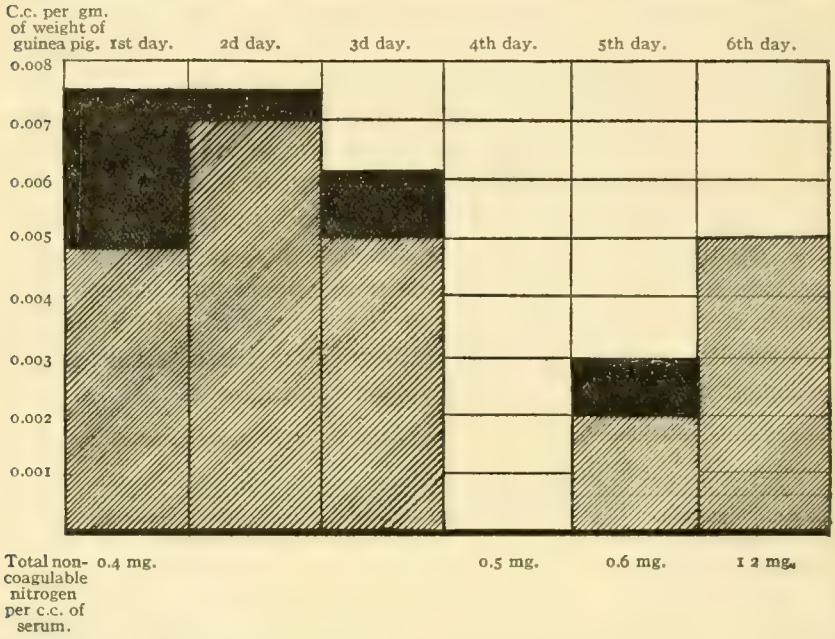
Dose.	Weight of guinea pig.	Dose per gm. of weight.	Result.
3.2 c.c.	320 gm.	0.01 c.c.	Death in 2 min. Lungs emphysematous. Heart blood fluid. Heart beats strongly.
1.6 c.c.	310 gm.	0.005 c.c.	No effect.

Typical anaphylactic shock was produced with immediate fatal result in a guinea pig which had not been sensitized. This result is of course not comparable to the work of Bruck (4) and Friedberger and Ito (5), who have attempted to show that animals may be sensitized to iodized proteins. It is interesting to note that von Dungern and Hirschfeld (3) observed that their iodized sera underwent autolysis readily, but they could find no explanation for this fact, because they found no difference in the digestion of casein when iodized. They entirely overlooked the fact that the iodin might act on other bodies besides proteins. We have observed no increase in autolysis if an excess of iodin is present because the iodin

itself interferes with ferment action. The serum proteins are, however, digested more easily by trypsin after such treatment. We have been unable to secure toxic sera by treatment with strong hydrogen peroxide.

INFLUENCE OF TIME ON CHLOROFORM EXTRACTION.

We next determined the length of time required for serum kept at room temperature under chloroform to become toxic. For brevity the result has been charted in text-figure 1. The serum before

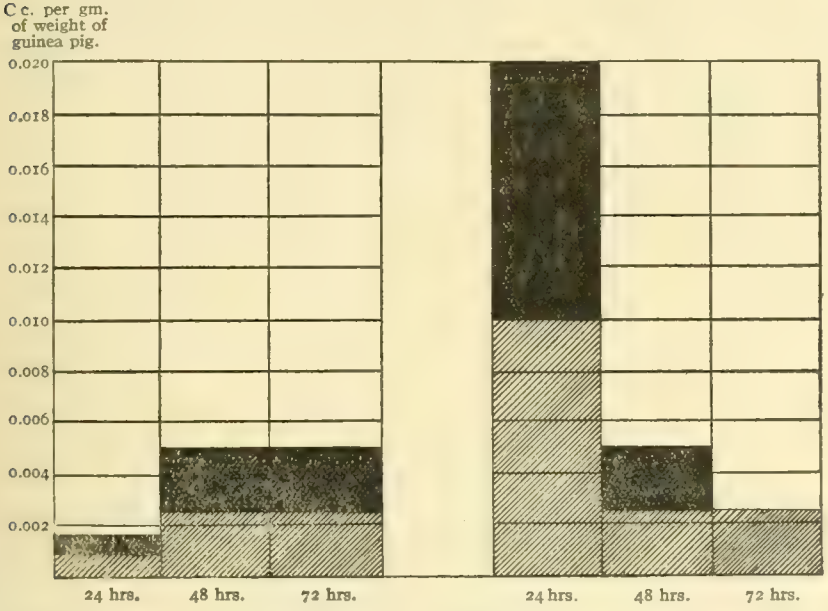


Black = minimum lethal dose per gm. of weight of guinea pig.
Shaded = sublethal dose.

TEXT-FIG. 1. Toxicity of guinea pig serum treated with chloroform at room temperature.

treatment was not toxic in large doses (5 c.c.), and the total non-coagulable nitrogen per cubic centimeter amounted to 0.2 of a milligram. As will be seen from the chart there was a progressive increase in toxicity after the first day until a maximum was reached on the fifth day, when the minimum lethal dose was 0.003 of a cubic centimeter per gram of weight, or 0.75 of a cubic centimeter for a guinea pig weighing 250 grams. There then follows a rather

sudden loss of toxicity, so that 0.005 of a cubic centimeter per gram of weight was no longer toxic. During this time there had been a constant autolysis of the serum, as indicated by the increase in the total non-coagulable nitrogen (Folin's method) from 0.2 of a milligram per cubic centimeter to 1.2 milligrams per cubic centimeter on the sixth day. In each case the minimum lethal dose caused the typical anaphylactic complex, although it was found that excessive

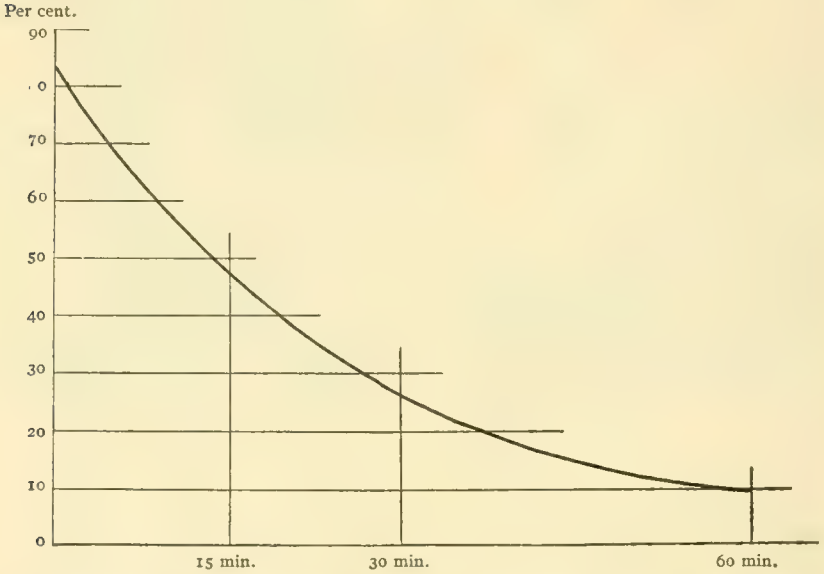


TEXT-FIG. 2. Toxicity of guinea pig serum treated with chloroform at 37° C.

doses, two minimum lethal doses, caused thrombosis in the larger vessels and heart.

When left at 37° C. the serum becomes toxic much earlier, and the atoxic stage is correspondingly produced at an earlier period of time (text-figure 2). The active serum so prepared has reached a very toxic stage in twenty-four hours, so that 0.0015 of a cubic centimeter per gram of weight of guinea pig is toxic,—0.31 of a cubic centimeter killed a guinea pig weighing 210 grams,—while the

inactivated serum (fifteen minutes at 54° C.) is toxic only in a much larger dose, 0.02 of a cubic centimeter per gram of weight. After forty-eight hours, however, the active serum has become less toxic, while the inactivated serum has become more toxic than before. Here, as in the previous experiment, there is a constant increase in the amount of non-coagulable nitrogen. In other experiments we have observed that the toxicity will reach its maximum within eight to twelve hours and will then rapidly decline. This depends entirely upon the amount and the rate of removal of the lipoid protective substances. Thus the inactivated serum becomes toxic less rapidly owing to the fact that the antitrypsin is removed more slowly than from the active serum at 37° C.



TEXT-FIG. 3. Curve showing the rate of extraction of serum antitrypsin by chloroform at 37° C.

The rate of removal of the antitrypsin from the serum by chloroform is shown in text-figure 3. In this experiment samples of serum were removed from the incubator at fifteen minute intervals, and the antitrypsin was determined by the method described in our previous papers (6).

0.075 c.c. of normal serum inhibited 84 per cent. of digestion.
After 15 minutes under chloroform it inhibited 48 per cent. of digestion.
After 30 minutes under chloroform it inhibited 27 per cent. of digestion.
After 1 hour under chloroform it inhibited 10 per cent. of digestion.

In one hour practically all the antitrypsin has been extracted. When made strongly alkaline this extraction is markedly accelerated, while the inactivated serum is more resistant to the chloroform extraction during the first half hour, although after about an hour's extraction the lipoids in this case are also removed. The serum is toxic, however, before all the antitrypsin is removed. This is shown by removing a sample after one half hour's incubation. These sera kill in a dose of about 0.01 of a cubic centimeter per gram of weight, and in this case the immediate cause of death is intravascular coagulation. There is no increase in the non-coagulable nitrogen at this time.

PROTOCOL I.

One Hour Chloroform Serum.

Dose.	Weight.	Dose per gm. of weight.	Result.
4.5 c.c.	300 gm.	0.015 c.c.	Immediate death.
3.2 c.c.	270 gm.	0.012 c.c.	Respiratory spasms. Prostrated for 30 min. Recovery.

Two Hour Chloroform Serum.

4.0 c.c.	260 gm.	0.015 c.c.	Immediate death.
1.6 c.c.	215 gm.	0.0075 c.c.	Death in 15 min. Lungs distended. Heart blood fluid. Heart beats.

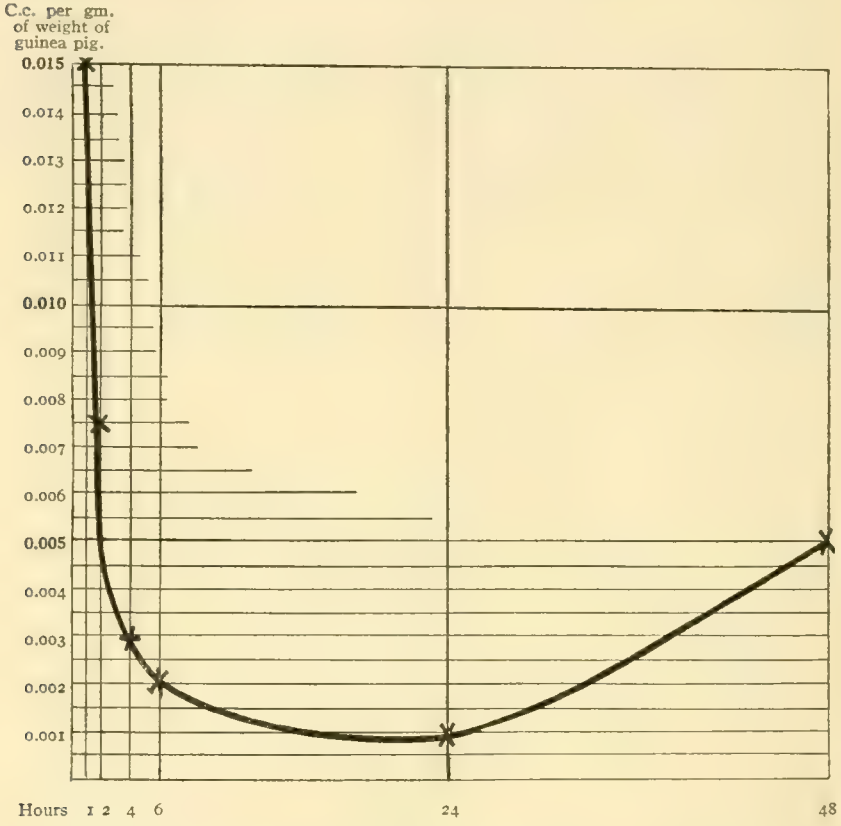
Four Hour Chloroform Serum.

1.7 c.c.	280 gm.	0.006 c.c.	Immediate death. Heart blood clotted. Heart does not beat. Lungs emphysematous.
1.0 c.c.	340 gm.	0.003 c.c.	Death in 30 min. Heart beats. Lungs somewhat distended.

Six Hour Chloroform Serum.

1.1 c.c.	370 gm.	0.003 c.c.	Immediate death. Heart blood clotted. Lungs distended.
0.62 c.c.	310 gm.	0.002 c.c.	Immediate respiratory spasms. Prostrated. Death in 20 min. Small clot in right heart. Blood fluid. Lungs slightly distended.

In text-figure 4 is shown the rate of increase of toxicity observed at 1, 2, 4, 6, 24, and 48 hour intervals of serum kept under chloroform. As will be observed there is a rapid increase during the first six hours, after which the toxicity remains at a constant



TEXT-FIG. 4. Curve showing the increase of toxicity of guinea pig serum treated with chloroform at 37° C. Minimum lethal dose in c.c. per gm. of weight.

level for a period of twelve or more hours, and then rapidly decreases. The minimum lethal doses have been charted from protocol I. The twenty-four and forty-eight hour tests were made at a different time, though with the same lot of serum.

INTRAVASCULAR CLOTTING.

The minimum lethal dose of the toxic sera when given intraperitoneally is much larger, at least five to six times the intravenous dose being required. The immediate cause of death observed in the minimum lethal dose has been either a typical anaphylactic complex, with the lungs emphysematous, the heart beating, and the blood fluid,—observed in the acidified and filtered serum, in the iodized serum, and in the chloroform serum, especially when kept at room temperature; or death has been due to immediate intravascular coagulation with a secondary emphysema, observed in the ether sera, and in the chloroform serum prepared at 37° C. In order to rule out the effect of coagulation we have made the following experiments. The serum used in this experiment had been incubated for two hours at 37° C. The minimum lethal dose was then determined.

Dose.	Weight of guinea pig.	Dose per gm. of weight.	Remarks.
1.0 c.c.	340 gm.	0.003 c.c.	Weak. Prostrated. Recovered.
1.1 c.c.	220 gm.	0.005 c.c.	Immediate death. Intravascular coagulation.

Some of this serum was mixed with saline solution to which had been added 1 per cent. sodium citrate, and injected.

Dose.	Citrate.	Weight of guinea pig.	Dose per gm. of weight.
1.25 c.c.	1.25 c.c.	250 gm.	0.005 c.c.

Immediate death. Heart found beating. Lungs emphysematous. Small clot in right heart.

Inasmuch as there was a small clot present we increased the amount of citrate.

Serum.	Citrate.	Weight of guinea pig.	Dose per gm. of weight.
1 c.c.	2 c.c.	200 gm.	0.005 c.c.

Death in 2 min. Blood fluid. Lungs emphysematous. Heart beating.

Even without a trace of clotting, we find the serum prepared in this way toxic in the same dose, and post-mortem examination shows a typical lung finding.

We next prepared some hirudinized animals and injected them five minutes after treatment with hirudin. In this case each guinea pig received two minimum lethal doses of the toxic sera.

Dose.	Weight of guinea pig.	Amount of serum per gm. of weight.
2.25 c.c.	225 gm.	0.01 c.c.

Typical death in two minutes. No clotting. Lungs emphysematous.

We then used a single minimum lethal dose and also one and one fourth minimum lethal doses, as will be seen below.

Dose.	Weight of guinea pig.	Dose per gm. of weight.
0.825 c.c.	165 gm.	0.005 c.c.
1.0 c.c.	160 gm.	0.0062 c.c.

No immediate result. The animals seemed well and were placed in the cage over night. The next morning both were found dead; in each case the blood was fluid, the lungs emphysematous, although not so markedly as in acute anaphylactic shock.

INHIBITION OF TOXIC ACTION.

Dilution of a single minimum lethal dose with an equal volume of normal guinea pig serum, and incubation for thirty minutes before injection does not alter the toxicity, but heating to 70° C. for thirty minutes greatly decreases it.

If the assumption that we are removing a protective substance from the serum is correct, then by returning the extracted substance to the serum it should become non-toxic. We have previously shown (1) that by saponifying the ether and chloroform extracts of sera we can recover all the antitrypsin. These protective lipoids are not originally present in the serum as soaps, but inasmuch as it is practically impossible to get the lipoids back into solution in the serum in the fine state of dispersion in which form only they are active, we must saponify them, and in the form of soaps attempt to reintroduce them into the serum. Twenty cubic centimeters of guinea pig blood were placed under chloroform for two hours, the serum was then separated and tested for toxicity.

Dose.	Weight of guinea pig.	Dose per gm. of weight.	Result.
0.9 c.c.	180 gm.	0.005 c.c.	Immediate death with intravascular clotting, etc.
0.51 c.c.	170 gm.	0.003 c.c.	Scratches; no symptoms.

The minimum lethal dose was therefore 0.005 of a cubic centimeter per gram of weight. The chloroform extract was mixed with an equal volume of alcohol and water and made strongly alkaline with sodium hydrate. This was heated in the water bath for an hour. The mixture was then acidified, and the fatty acids were

taken up in ether and resaponified by neutralization with alcoholic sodium hydrate. These soaps were now mixed with 1.5 cubic centimeters of the serotoxin and incubated for fifteen minutes.

Dose injected.	Weight of guinea pig.	Dose per gm. of weight.
1.1 c.c.	215 gm.	0.005 c.c.

There were no immediate symptoms and the animal remained well. The toxicity had therefore been completely neutralized. In like manner one can completely neutralize the serotoxin by adding soaps of the unsaturated fatty acids, which, as we have shown (6), have marked antitryptic properties, although they are less active when mixed with serum than when dissolved in salt solution. The serotoxin here used was freshly prepared and autolysis had not occurred, so that preformed toxic split products of proteins (primary proteoses) were probably not present. If on the other hand we use a serotoxin which has been prepared at room temperature by standing for several days, in which there is a marked increase in the total non-coagulable nitrogen, and from which one can isolate toxic proteoses, then the neutralization by the soap is no longer complete, the effect depending of course on the relative amount of the proteins and their toxic split products already formed.

A chloroform serum standing for five days at room temperature caused immediate death in doses of 0.003 of a cubic centimeter per gram of weight. Soap was prepared from serum lipoids and mixed with the dose.

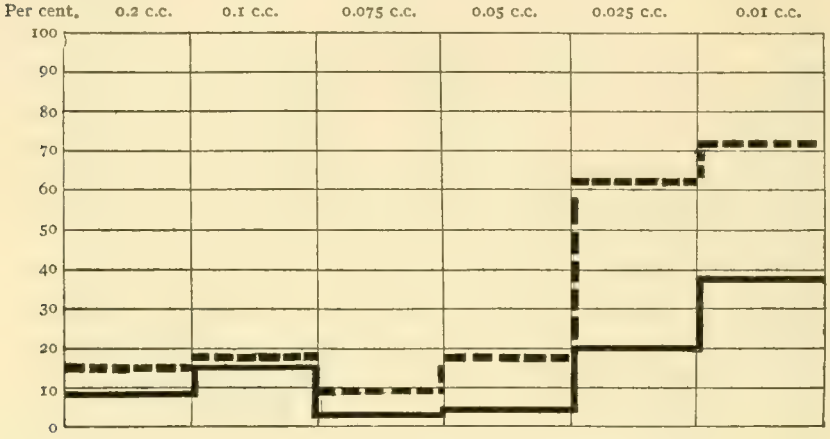
Dose.	Weight of guinea pig.	Dose per gm. of weight.
1 c.c.	280 gm.	0.0035 c.c.

There were immediate respiratory convulsions, after which the animal recovered for ten minutes; then clonic convulsions occurred at frequent intervals, with death in thirty minutes. In a similar way a dose was prepared by adding 10 mg. of linseed soap (iodin value 110), and in this case, also, while immediate death was prevented, the ultimate result was fatal.

Dose.	Weight of guinea pig.	Dose per gm. of weight.
1 c.c.	330 gm.	0.003 c.c.

If the toxicity of the serotoxin depends on the exposure of the proteins, so that they can be easily split to toxic products in the injected animal, then the reduction of the normal antiferments in the injected animal should render the serotoxin more toxic than for a normal animal. A three hour serotoxin (chloroform at

37° C.) was used, the minimum lethal dose of which was 0.005 of a cubic centimeter per gram of weight. Four guinea pigs were prepared in the morning by shaving the abdomen and painting with tincture of iodin, so that each animal received about 0.2 of a gram of iodin. In this way one can reduce the serum antitrypsin in an amount shown in text-figure 5, in which the black line represents the



Black line = before treatment (A. M.).
Dotted line = after being painted with tincture of iodin (P. M.).
TEXT-FIG. 5. Effect of iodin on antitrypsin of guinea pig.

amount of digestion of casein in the presence of varying amounts of the guinea pig serum drawn in the morning before treatment, and the dotted line represents the digestion in the serum of the same guinea pig in the afternoon. This decrease in antitryptic activity is probably due to the saturation by the iodin of the unsaturated carbon bonds of the fatty acids, as we have stated elsewhere (1).

A sublethal dose was first injected into an iodized guinea pig.

Amount of serum.	Weight of guinea pig.	Dose per gm. of weight.
0.54 c.c.	180 gm.	0.003 c.c.

Immediate death. Heart blood clotted. Lungs slightly distended. Gall bladder markedly distended.

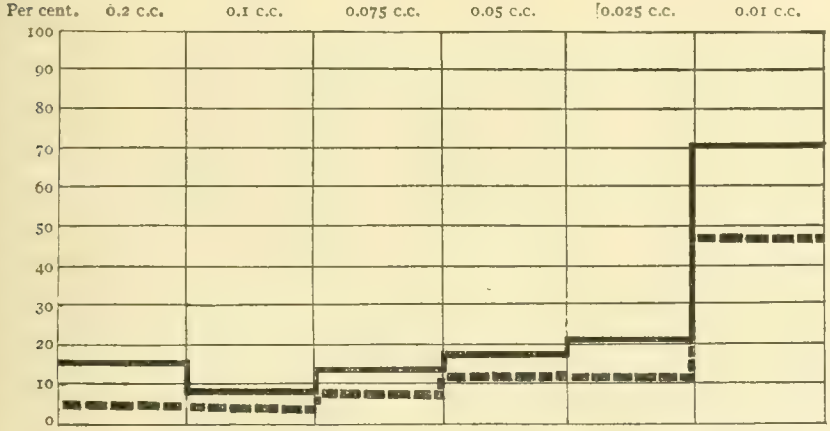
Next a half sublethal dose was given.

Amount of serum.	Weight of guinea pig.	Dose per gm. of weight.
0.27 c.c.	185 gm.	0.0015 c.c.

The animal was immediately prostrated, sick, with occasional convulsions. Death in thirty minutes. Heart beating. Blood fluid. Lungs slightly distended.

The experiments indicate that the toxicity of the serotoxin is greater for animals in which the antitrypsin has been reduced, thus permitting the ferments normally present to be more active. A single injection of a sublethal dose, given intravenously or intraperitoneally, increases the antitrypsin in the guinea pig; the effect can be demonstrated within twenty-four hours, and persists for at least two weeks. A similar increase has been noted following anaphylaxis (Meyer (7), Pfeiffer (8, 9)). Zinsser (10) has recently noted an increased resistance to a minimum lethal dose of a strong anaphylatoxin prepared from typhoid bacilli, following a single sublethal dose given seven to twelve days previously.

If the serotoxic effects are due to an intravital splitting of the serum protein, then during the period of increased antitrypsin fol-



Black line = original antitrypsin of guinea pig serum drawn on Feb. 24, 1914.
Dotted line = serum drawn on Feb. 28, 1914.

TEXT-FIG. 6. Increase of antitrypsin following a single injection of a sublethal dose of serotoxin.

lowing a sublethal dose there should be an increased resistance to the serotoxin. The effect of a sublethal dose on the guinea pig antitrypsin is shown in text-figure 6. The black line indicates the percentage of casein digestion as influenced by the normal guinea pig serum drawn on February 24, 1914. A sublethal dose of serotoxin was given intraperitoneally on the same day. A second

sample of blood was drawn on February 28, and is shown in the dotted line. As will be seen, there is a well marked increase in inhibiting power.

The increased resistance to serotoxin is shown in the following experiment (February 24).

Dose of serotoxin.	Weight of guinea pig.	Dose per gm. of weight.
1.9 c.c.	190 gm.	0.01 c.c.
Guinea pig sick and prostrated.	Complete recovery.	

The following day the guinea pig received 0.006 of a cubic centimeter, per gram of weight, of a serotoxin preparation of which the minimum lethal dose was 0.005 of a cubic centimeter. There were no symptoms. After several days following the original sublethal dose we have secured animals that are resistant to two minimum lethal doses.

EXPERIMENTS WITH PURIFIED HORSE SERUM ALBUMEN.

In order to compare the results which we had so far obtained with a pure protein intoxication we prepared some horse serum albumen in the following manner: The globulins of the serum were thrown out of solution by half saturation with ammonium sulphate, and after filtration, the albumens were removed from the filtrate by precipitation with acetic acid. This precipitate was redissolved in water made slightly alkaline with sodium carbonate, treated with an equal amount of a saturated solution of ammonium sulphate, filtered, and the filtrate again treated with acetic acid. The final precipitate was dialyzed against running water until free of acid and ammonium sulphate, and was then evaporated to dryness. It was made up in a 1 per cent. solution in normal saline. Of this solution a dose of 0.02 of a cubic centimeter per gram of weight was not toxic for the guinea pig. On testing its antitryptic property it was found that two cubic centimeters of such a solution inhibited 86 per cent. of tryptic digestion, and on digesting two cubic centimeters with a strong trypsin solution for forty-five minutes, only 0.1 of a milligram of non-coagulable nitrogen was obtained. If we now extract the solution by mixing with chloroform at 37° C. for two hours we find the toxicity well marked in the dose which was not at all toxic for the unextracted serum.

Amount of serum.	Weight of guinea pig.	Dose per gm. of weight.	Result.
4 c.c.	200 gm.	0.02 c.c.	Immediate death. Heart blood clotted. Lungs distended.
2 c.c.	190 gm.	0.01 c.c.	No effect.

After extraction for twenty-four hours the toxicity had still increased.

Amount of serum.	Weight of guinea pig.	Dose per gm. of weight.	Result.
2.4 c.c.	240 gm.	0.01 c.c.	Killed in 2 min. Heart blood clotted.
0.9 c.c.	180 gm.	0.005 c.c.	No effect.

The last dose, which was sublethal, was now injected into an iodized guinea pig. The method of preparing the guinea pig has been already described.

Dose.	Weight of guinea pig.	Dose per gm. of weight.
1.1 c.c.	220 gm.	0.005 c.c.

Immediate death. Heart blood fluid. Lungs distended. Gall bladder distended.

As with the serotoxin, there was a lessened resistance to the toxic effect in the iodized guinea pig. When the antitryptic effect of the extracted (twenty-four hour) horse serum solution was tested, it was found that it now inhibited only 33 per cent. of casein digestion, and was itself digested by trypsin much more easily (0.35 of a milligram for 2 cubic centimeters of solution). After extraction for seventy-two hours the toxicity had increased so that a dose of 0.005 of a cubic centimeter per gram of weight caused marked toxic symptoms but did not kill. At this time the serum albumen was no longer antitryptic. Inasmuch as there are no ferments present in the serum albumen solution, and no trace of autolysis can be obtained when such solutions are incubated, there is no preformation of toxic split products, and whatever increase in toxicity occurs must be due to the increased availability of the serum albumen for attack by the ferments of the injected animal.

In the serotoxin we have a somewhat different condition due to the presence of quite active proteolytic ferments. It would therefore seem reasonable that the ferments would attack the serum proteins as soon as the antitrypsin had been completely removed, and

produce toxic split products,—primary proteoses. This condition actually obtains. If one fractionates a serotoxin which has been under chloroform at 37° C. for only two hours, only faint traces, or no evidence at all, of primary proteoses can be obtained. From twenty-four hour serotoxin we have obtained toxic proteoses, as is shown in the following experiment.

Ten cubic centimeters of normal guinea pig serum were mixed with chloroform and incubated for twenty-four hours. The chloroform was then removed, the native protein removed from the serum by the heat and acid method, and the filtrate made faintly alkaline to throw out the acid albuminates, and again filtered through kaolin. The primary proteoses were then precipitated by half saturation with ammonium sulphate. These were removed, dialyzed until free from all traces of ammonium sulphate, and then made up to the original volumes. When injected intravenously in 0.1 per cent. salt solution the primary proteoses are toxic in a dose of about 0.01 cubic centimeter per gram of weight. When so prepared the toxicity is unaltered by boiling.

DISCUSSION.

In the preliminary experiments we observed the marked similarity to anaphylactic death which our animals showed on the injection of the serotoxin, and later, while working with the rapidly prepared chloroform sera, the paradoxical similarity to cytotoxic death—immediate coagulation—was noted. In view of the fact that the usual method of death is intravascular coagulation, we have used the term serotoxin to designate the entire toxic effect of the extracted sera, although we wish to emphasize the importance of the protein cleavage phenomena.

The toxicity of organ extracts first noted by Brieger and Uhlenhuth (11), was shown by Blaizot (12), Gley (13), and by Dold and Ogata (14) to depend on the intravascular coagulation, and could be rendered completely inert by the treatment of animals with hirudin. Ichikawa (15) has recently shown that sodium citrate will also render a toxic dose non-toxic. Serotoxin differs fundamentally from the cytotoxin in that the toxic effects persist when coagulation is prevented by hirudin or citrate. The toxicity is due to

factors quite apart from the altered coagulation balance, although we believe that it will be found that the unsaturated lipoids play an important part in the mechanism of coagulation and its prevention.

From our experiments we believe that we are justified in concluding that the exposed homologous serum proteins are capable of being split when injected into an animal, and produce the observed toxic effect. In many of our experiments the exact picture of acute anaphylactic death was observed; in sublethal doses a marked fall in temperature and extreme prostration were noted, the latter being most marked in sublethal doses of sera which had been permitted to autolyze. The toxicity of serum protein is well illustrated in the experiment with purified horse serum albumen which was not toxic in doses of 0.02 of a cubic centimeter of a 1 per cent. solution per gram of weight, but when extracted with chloroform it gradually became more and more toxic until a final dose of 0.005 of a cubic centimeter per gram of weight (or 0.00005 of a gram of serum albumen per gram of weight) was sufficient to cause marked toxic effects. The length of time required to extract the lipoids from this solution indicates how intimate the relation of the lipoid to the serum albumen complex must be.

That the exposed serum proteins are the substances which produce *in vivo* the toxic effects when split, is shown by the fact that in the rapidly prepared serotoxin no increase in non-coagulable nitrogen is demonstrable, and that no toxic split products (primary proteoses) can be isolated by fractioning the serum proteins at this time. The antiferments having once been extracted, there is of course no further impediment to ferment action, such sera become actively proteolytic, as was first shown by Delezenne and Pozerski (16), and undergo autolysis as shown by the marked increase in the non-coagulable nitrogen. Under these conditions we have always been able to isolate preformed toxic split products. The effect of the soaps, too, in not completely neutralizing the serotoxin at this period is confirmatory evidence, since the soaps act by preventing the splitting of native proteins (true antitryptic effect, rather than anti-ereptic). With continual autolysis we should expect lessened toxicity, and this is found to be the case. That the

normal inhibitory mechanism of the serum markedly influences the intravital splitting, as it does in the test-tube, is shown by the great increase of toxicity of the serotoxin for animals with lessened antiferments and by the greater resistance of animals with increased antiferments. We believe that the effect of the iodin is due to a saturation of the unsaturated bonds of the fatty acids of the lipoids, rendering the substituted product less active as an antitryptic agent. The effect of a single sublethal dose of serotoxin in increasing the antiferment can possibly be explained by a lowered oxidation of the organism due to the toxin, with a resulting lowering of the physiological oxidation of these unsaturated compounds, or it may be due to a breaking up by the toxic action, of protein-lipoid combinations with a resulting increase in the lipoidal elements in the blood. From our studies we are even more firmly convinced of the biological importance of the protein-lipoid combination in the organism, and the correlated necessity of according to lipolytic action greater emphasis in the part of the adjunct which breaks up these combinations before actual proteolytic changes can take place. The small amount of purified horse serum albumen (after complete chloroform extraction) required to kill a non-sensitized animal is significant from this point of view.

A large number of investigators, notably Vaughan (17), Friedberger (18), and Pfeiffer (9), have, during the past few years, emphasized the importance of the split products of proteins in the production of the toxic effects in the various infections, but the basic idea has always been held that the substrate which was acted upon was the foreign protein concerned, and that the homologous proteins might be neglected, although the work of Keysser and Wassermann (19), Doerr (20), Bordet (21), and Ritz and Sachs (22) indicate that adsorption phenomena and colloidal changes might render homologous sera toxic. The idea, however, that the adsorbed substances were the antiferments, or protective agents, has not been expressed, and no efforts have been made to remove such antiferments, possibly because of the unfortunate view that the antiferments represented immune bodies or split products of the protein molecule.

From a clinical point of view we are inclined to believe that the

proof of the toxicity of exposed serum proteins may be of importance. Longcope (2) has recently shown that repeated injections of heterologous proteins can cause marked nephritic changes. The profound prostration shown by leukemics in the terminal stages when the serum ferments are known to increase greatly, so that such sera may become proteolytic, suggests the profound prostration observed in experimental animals injected with sublethal doses of autolyzed sera. Further experiments are now in progress to determine the results from chronic sero-intoxication.

CONCLUSIONS.

1. Sera from which the protective lipoids (unsaturated fatty acids) have been removed are toxic for the homologous animal.

2. The toxicity is due to three factors: (a) an alteration in the mechanism of coagulation, with resulting intravascular coagulation; (b) the exposure of the native serum proteins; (c) the formation of toxic split products (primary proteoses) by autolysis.

3. A definite maximum of toxicity can be determined, with a final stage of atoxicity due to continued autolysis.

4. Hirudin and sodium citrate do not protect animals.

5. Heating to 70° C. destroys, or greatly lessens, the toxicity of the serotoxin, although the isolated proteoses are toxic after boiling.

6. The return of the extracted lipoids (saponified) neutralizes the toxicity.

7. Unsaturated soaps also neutralize the toxicity.

8. Sublethal doses produce extreme prostration, marked fall in body temperature, no eosinophilia, and an increase of antitrypsin.

9. Sublethal doses of rapidly prepared chloroform sera cause a decrease in coagulation time; sublethal doses of autolyzed sera cause an increase in coagulation time.

10. Previously injected animals are more resistant (increased antiferments).

11. Iodized animals are less resistant (decreased antiferments).

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Further notes on the genus Microcotyle.

By

G. A. MacCallum, M. D., New York.

With 4 figures in the text.

As stated in a previous paper in this journal (Vol. 34, 1913, Syst., p. 223), one is impressed in studying the Microcotylidae with the lack of variety in the arrangement and form of the internal organs so that the differentiation of species is dependent largely upon outward differences. Of these the number, form and structure of the caudal suckers and the peculiar armature of spines which often occurs about the genital opening are perhaps the most constant and characteristic features, although of course there are many other things which must be taken into consideration in deciding this point. In the table which follows and which is a continuation of that given in the previous paper, it is attempted to give these data as accurately as possible, and it is hoped that this basis for classification will be adopted by helminthologists in the case of this genus to avoid confusion.

4 new species are added to the genus as the result of the study of the parasites of the gills of marine fishes obtained partly from the New York Aquarium, partly in the laboratory of the U. S. Fish Commission at Woods Hole and partly in the fish markets of New

York. In the course of these studies it has seemed pretty well arranged by Nature that the parasites of the gills of fish are placed there according to families — for instance the Microcotylidae are seldom or never found on the same fish as the Octocotylidae or Acanthocotylidae. At least it is my experience that in such a family of fish as the Salmonidae the parasites on the gills will almost invariably be found to be Octocotylidae and so on.

Microcotyle macroura.

In the previous paper mentioned, a description of this form was given which, owing to insufficient material, was uncertain or incomplete in places. Since that time study of new material has made it possible to confirm or complete some of these observations. The description of the mouth, its suckers, and of the peculiar position of the small pharynx-like structure on the dorsal surface of the digestive canal is all correct. There is a rather twisted or tortuous canal penetrating the pharynx but it seems that there is also a freer and unguarded opening from the mouth directly into the digestive tract.

The intestinal coeca are extraordinarily widely ramified through the body, their diverticula opening on both sides of the main line of the channel and extending outwardly quite to the skin. Among these diverticula and in very close contact with them lie the other organs. There is hardly any lining to be made out even with high powers. The epithelial cells must be extremely thin and flat for they can nowhere be clearly discerned.

The parenchyma throughout except in the ventral part of the caudal disc is composed of large polygonal cells with relatively minute nuclei. In the caudal disc the parenchyma is largely replaced by muscle and what cells are present fill elongated spaces among these fibres.

The genitalia are precisely as described before but with the new material it was possible to trace in serial sections the continuity of the vitelline ducts with the two vaginal tubes which join as they approach the vaginal orifice and pass through a thick muscular mass to open on the dorsal surface.

In two reconstructions of the genitalia from these new serial sections the duct figured at *K* in fig. D and designated as possibly the Canalis genito-intestinalis could be traced out precisely as

it is shown there and could be shown to end blindly in the parenchyma and not in the intestine. It seems to contain spermatozoa and must therefore be put down definitely at the Receptaculum seminis. Otherwise the female genitalia proved to have been correctly described and sketched in that paper.

The only other point of interest is that the W-shaped arrangement of chitinous rods about the cirrus opening is found to be a kind of chitinous skeleton for the muscular cirrus itself. The stouter anterior parts of these rods pierce the muscular sac obliquely and come into immediate connection with the inner protrusible tube. Outside they run quite far back and disappear near the Vas deferens. They are not so clearly outlined as it seemed from the previous material but give off twigs and branches as various places.

Microcotyle pogoniae n. sp.

The worm occurs on the gills of the common sea drum, *Pogonias cromis*, sometimes in such great numbers as to menace the life of the host.

The body is elongated with a large sucker disc which constitutes about one-third of the total length and which at its upper end is sharply marked off by indentations which give it almost the form of an arrow head. There are about one hundred and eight suckers which reach their greatest size at about the middle of the disc starting above with two small ones and again decreasing in size toward the caudal termination (Fig. A a).[1])

The chitinous skeleton is quite delicate and has the arrangement shown in Fig. A e. The suckers at the mouth are large and armed at their margins by a row of minute spicules. Their cavity is crossed by a partition separating it into two loculi the inner and larger of which is deeply indented at its median border (Fig. A c). Immediately posterior to these in the mid line is the peculiarly shaped pharynx which has a narrow anterior part and a more bulbous portion (Fig. A d). A short way behind this the esophagus divides into the two lateral coeca which run far back into the caudal disc.

Directly behind the bifurcation of the intestine lies the genital pore which is unarmed and small in proportion to the size of the

1) Explanation of Abbreviations see pag. 402.

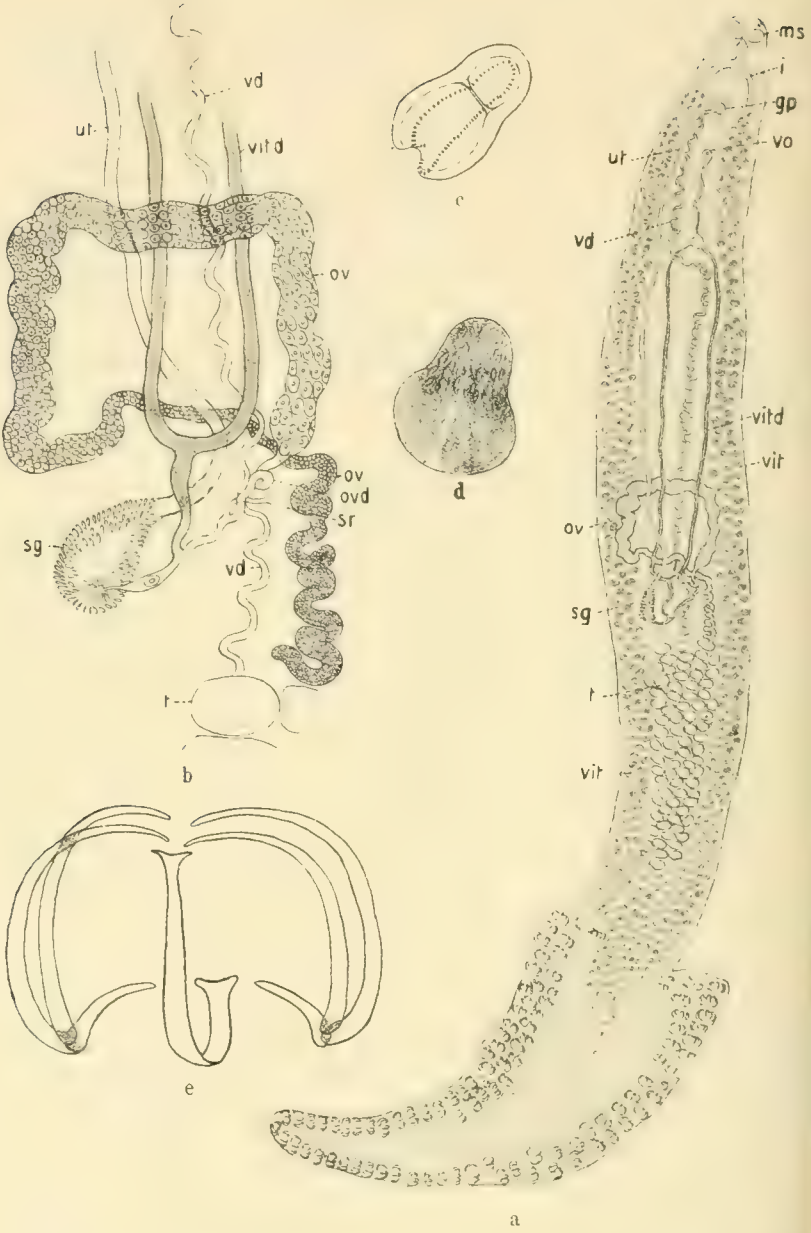

Fig. A. a *Microcotyle pogoniae n. sp.* b Detail of genital organs. c Mouth sucker. d Pharynx. e Chitinous skeleton of a caudal sucker.

worm. It lies about 0,4 mm behind the oral suckers. Behind it at a distance of about 0,65 mm on the dorsal surface is the sac-like orifice of the vagina which is also unarmed. From this there runs backward a tortuous tube which after forming an enlargement opens into two lateral vaginal canals which eventually become the lateral vitelline ducts. Posteriorly these begin to be filled with yolk material and enlarge until they reach the point at which they unite.

The ovary (Figs. Aa and b) is much longer and narrower and more convoluted than in most of the other species studied. It form first an irregular square across the middle of the worm and crossing to the left side coils irregularly backward toward the left where the eggs are most fully developed and the oviduct takes its origin. This duct proceeds backward to receive the common vitelline duct but gives off on the way a canal which runs to the left. The termination of this canal could not be made out and it is not shown in the drawing but it doubtless represents the seminal reservoir. Having received the vitelline duct the oviduct passes through the radiate shell gland into the bulbous uterus which then runs straight forward to the genital opening. The vitellaria are voluminous and extend from opposite the vaginal opening to the sucker disc, occupying both sides of the body. The testes, about 75 in number, extend nearly to the sucker disc, occupying the whole of the middle of the body from the termination of the uterus backward. The Vas deferens is large and tortuous and is usually filled with spermatozoa. It passes forward to open at the genital pore without the formation of any definite cirrus.

The eggs are yellow and fusiform with one rather rounded end. This is the posterior end and is not provided with a chitinous filament while the anterior end is pointed and extended into a very long tangled filament.

Measurements are as follows:

Length of worm: 12 to 13 mm.
Width: 1,25 mm.
Length and form of caudal disc: 4,5 mm triangular.
Average: 108 suckers.
Diam. of suckers: 0,2 × 0,15.
Testes: 75 in number.
Size: 0,10 to 0,15.

(Measurements continued)

Ova: 0,12 × 0,5 to 0,15 × 0,06.
Diam. across head: 0,45.
Cloacal aperture: 0,04 × 0,10.
Diam. of vaginal opening: 0,04 × 0,01.

Microcotyle carangis n. sp.

There were found on the gills of a yellow crevalle, *Caranx crysos* (Mitchill), 5 examples of a *Microcotyle* which differs very markedly from other species.

The worm is small, measuring only 4,4 mm by 0,8. The anterior end is expanded laterally and provided with a sucker at each angle of this expansion. The mouth opens directly into the comparatively small pharynx from which the short esophagus runs backward to divide into the lateral intestinal coeca which run dorsally toward the posterior extremity. A short distance behind the bifurcation is the cirrus sac which is pear shaped with a curious arrangement of spicules as shown in Fig. B a and Fig. B f. Two small clumps of hooklets are separate from the main group which lie in front of the muscular bulb of the cirrus. The Vas deferens can be plainly seen entering the posterior end of this bulb.

Behind this lies the female genital orifice which is also armed with spicules. The posterior end of the body forms the sucker disc which is hastate in form, one side being continued forward so as to make it asymmetrical. At the point there are on each side eleven small suckers with a chitinous skeleton formed as show in Fig. B d. At the extreme tip there are two strong hooks and between them, apparently to stiffen them, are two straight spines which extend almost as far posteriorly as the hooks, the whole being enclosed in a membrane (Fig. B e). On the right side of the body occupying the asymmetrical forward extension of the caudal disc there is a row of twenty-six much larger suckers which diminish in size as they near the body. These are of a quite different structure from any observed in other forms of *Microcotyle* as shown in Fig. B c. The chitinous skeleton is so asymmetrical in form as to give the sucker almost the appearance of some gamopetalous flower.

The ovary lies across the middle of the body as an irregular U-shaped structure placed with the convex side toward the head

(Fig. B b). The oviduct arises at its left end and passes backward toward the extremity of the uterus. On its way it is first joined

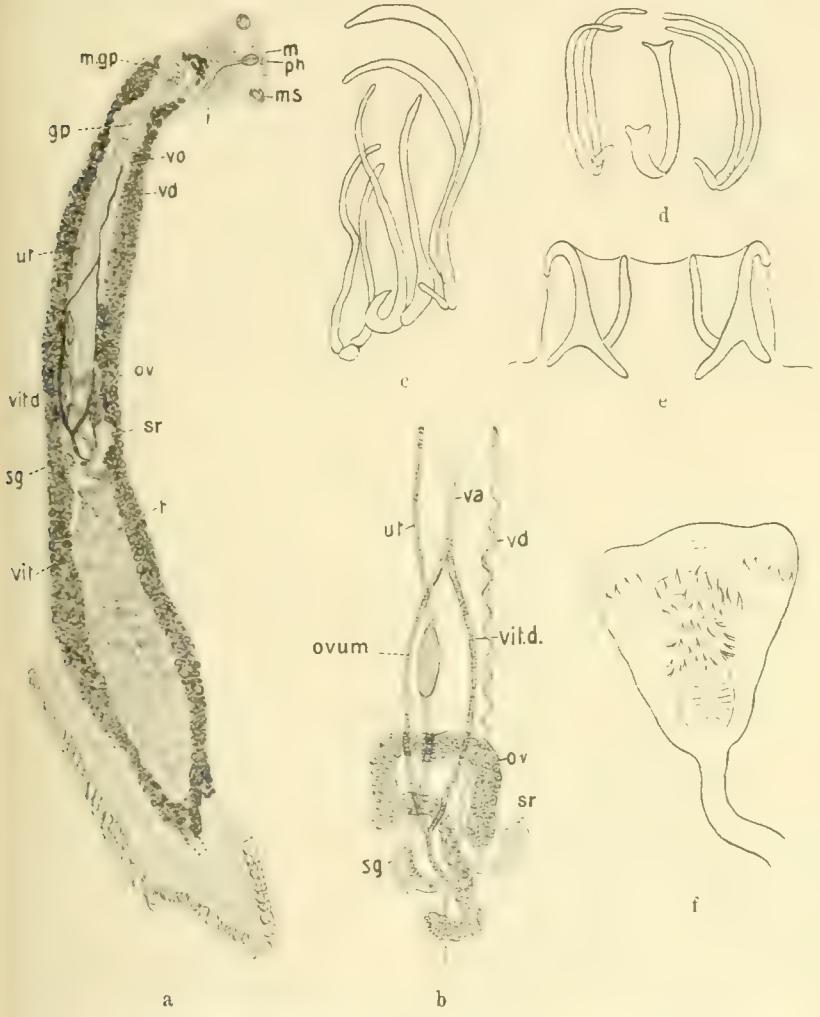

Fig. B. a *Microcotyle carangis n. sp.* b Detail of internal genital organs. c and d Chitinous skeleton of a caudal sucker. e Caudal spines. f Cirrus sac.

by the canal from a seminal reservoir which lies close to the end of the ovary and between it and the first row of testes. It next received the duct from the vitelline glands and then passes into

the ootyp which is surrounded by the shell gland. From this point the tube passes forward along the right inner margin of the vitellaria to the genital pore. The vitelline ducts, which unite as usual to enter the oviduct by the common tube described, again unite anteriorly to form the vagina which opens dorsally a short distance behind the genital pore. The vitellarium is very voluminous occupying the major part of the body on both sides. The eggs are yellow and fusiform with a chitinous filament at each end, the anterior being much larger than the posterior and very delicate and tangled near its end.

There are 60 testicles situated near the caudal end of the body from which the large tortuous Vas deferens runs forward to the muscular cirrus already described. The water vascular system is very distinct being composed of large ducts which proceed to the end of the disc receiving branches on their way from the surrounding tissues.

Measurements:

Length: 4,4 mm.
Width: 0,8 mm.
Ova: 0,11 × 0,03 mm.
Length and width of disc: 0,9 × 0,5 at base.
Suckers: 48 in number — larger ones 0,05 × 0,07; smaller 0,04 × 0,03.
Width of head: 0,47 mm.
Testes: 60 in number 0,07 mm.
Hooks at tip of disc: 0,029 mm long × 0,002 mm wide.
Spines at tip: 0,15 × 0,01 mm.
Oral suckers: 0,04 mm.
Hooklets around g. p.: 0,05 mm long.

Habitat: Gills of yellow Crevalle (*Caranx crysos*).
Location: Woods Hole, Mass. U. S. A.

Microcotyle angelichthys n. sp.

A worm closely related to others to be described but differing in one or two important particulars is found on the gills of the Angel fish, *Holacanthus ciliaris*, in numbers varying from a few to many thousands or enough to cause the death of its host.

It is small measuring 4.3 × 0,3 mm with a caudal sucker disc about 1 mm long armed with forty to sixty suckers (Fig. Ca). The

terminal mouth is flanked by suckers which have a central partition. The pharynx is large but rather delicate in structure opening into a short esophagus which divides in front of the genital pore. The genital pore itself is situated very near the anterior end of the body. It opens into a rounded sac thickly armed with spicules especially closely set round the aperture. There are two smaller groups of spicules placed laterally behind and the sac is surrounded by radiating muscle fibers (Fig. C b). The uterus and Vas deferens open into this sac, the latter through a delicate bulbous structure which appears to extend forward beyond the end of the uterus. The vaginal orifice lies behind and in the mid-dorsal line.

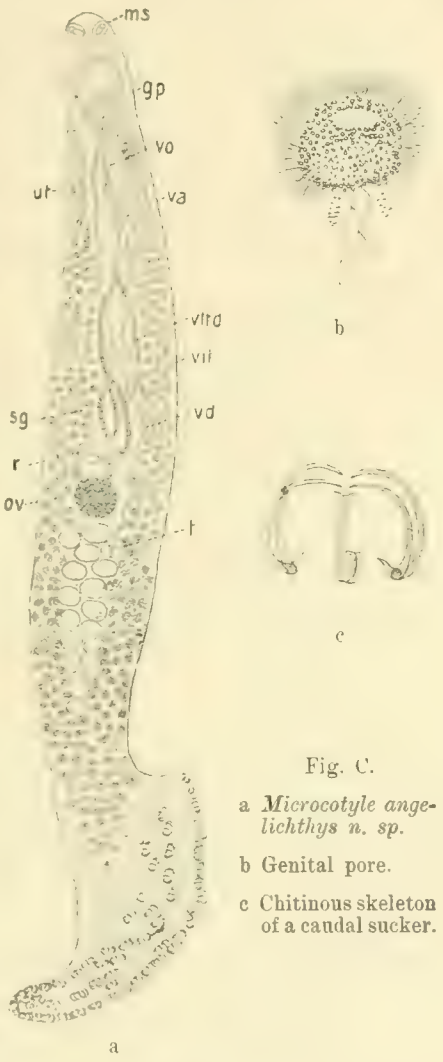

Fig. C.

a *Microcotyle angelichthys n. sp.*

b Genital pore.

c Chitinous skeleton of a caudal sucker.

One important respect in which this worm differs from the other Microcotylidae, is the fact that while in almost all of the others the ovary is placed across the middle of the body in the form of a long convoluted tube, in this case it is a more or less globular mass like that of the distomes placed on the right side just anterior to the testes. On the right side above this may be plainly seen the seminal reservoir and also its duct joining the oviduct. The shell gland is in the usual position behind or beside the tail of the Y-shaped vitelline reservoir. The oviduct is apparently given off the ovary toward the middle of the worm and receives the duct from the spermatheca and vitelline reservoir before entering the

beginning of the uterus. The uterus, surrounded here by a mantle of shell gland cells, makes its way upward toward the genital pore along the inside of the right vitellarium. The vitellaria are very plentiful and extend from nearly opposite the orifice of the vagina down each side caudad to almost the sucker disc.

The testes are relatively large but few in number, there being only about 18 or 20. The Vas deferens passes anteriorly on the left side. No eggs are seen in any of the specimens mounted so that at this writing no measurements of them can be given.

The suckers have the usual chitinous reinforcement, a drawing of which is given in Fig. C c.

The rest of the measurements etc. are given at the end of this description.

Measurements:

Length: 4,4 mm. Width: 0,3 mm.
Width of head: 0,12 mm.
Size of oral suckers: 0,5 mm.
Sucker disc: 1 mm long, 0,4 mm wide.
V. O.: Armed situated short distance posterior to g. p. and dorsal.
Number of suckers: 59 — 0,03 × 0,02.
Number of Testes: 20 — 0,06.

Habitat: Gills of *Holacanthus angelichthys*.
Locality: Aquarium, New York.

Microcotyle archosargi n. sp.

This form is to be found on the gills of the marine Sheep's head, *Archosargus probatocephalus*, and is so delicate as to be almost invisible when alive and in situ. It measures 8 by 0,8 mm and is much elongated, becoming very slender just in front of the asymmetrical spreading caudal disc (Fig. D a).

The mouth is terminal and supplied with the usual lateral suckers which are provided with a marginal membrane and subdivided by a distinct partition. The delicate pharynx passes into a short esophagus which gives rise as usual to two intestinal rami.

The common genital pore is surrounded by spicules which are arranged as shown in the figure (Fig. D c). Almost immediately behind this on the dorsal surface lies the opening of the vagina, also surrounded by an armature of spicules.

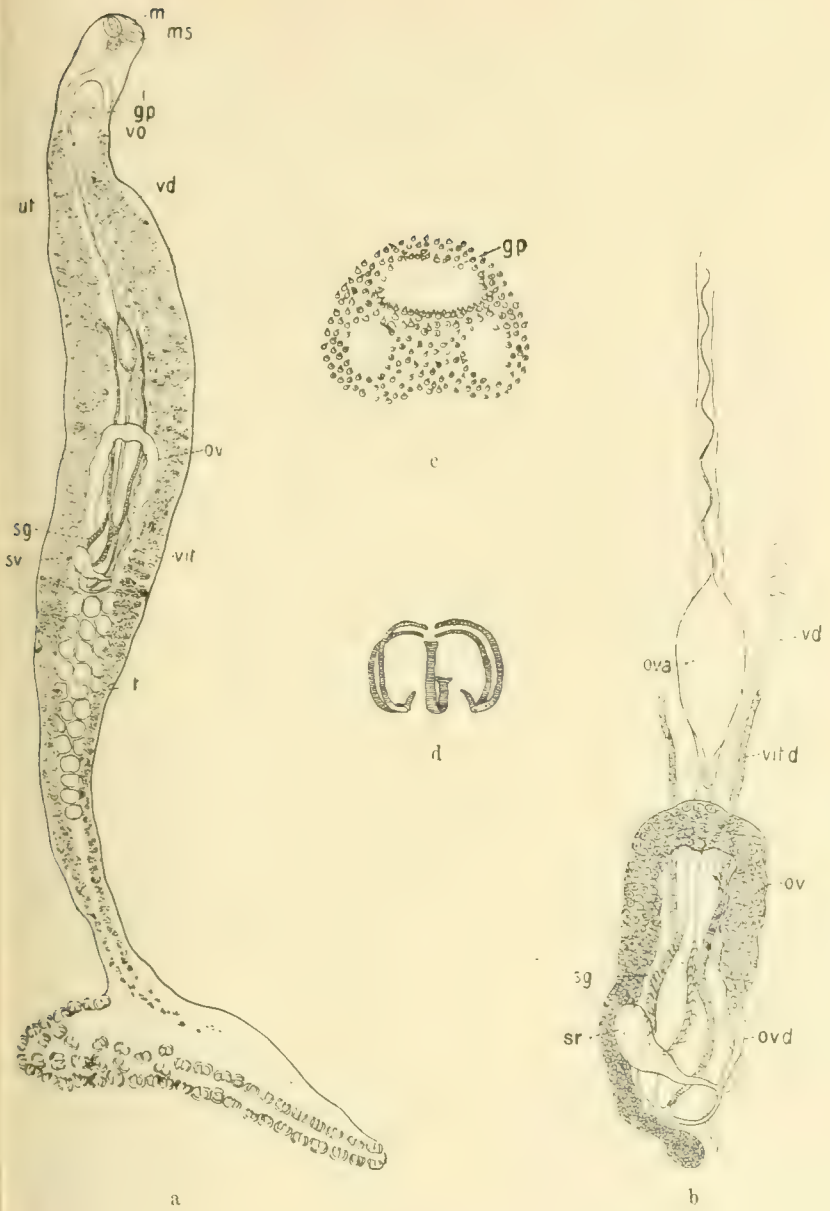

Fig. D.

a *Microcotyle archosargi n. sp.* b Detail of internal genital organs. c Armature of genital pore. d Chitinous skeleton of a caudal sucker.

The ovary (Fig. D b), in the form of a long bent tube lies across the body at about its middle, giving off the oviduct at its left end. This passes backward receiving the duct from the clubshaped seminal reservoir and then the conjoined canal from the vitelline glands until it reaches the shell gland which in this case extends dorsally before the uterus changes its course toward the genital pore.

The eggs, of which 3 were to be seen in one specimen are relatively large, have a very long tangled filament at each end, in which respect they differ from those of some closely related forms.

The testes are large and number from 20 to 35. The Vas deferens is tortuous and ends in the cloaca without any evident muscular cirrus. The caudal disc bears about one hundred and six suckers in a simple row along its margin. These are all alike and there are no hooklets or spines. Their chitinous skeleton is not especially characteristic and is shown in Fig. Dd.

Measurements:

Length: $8 \times 0{,}8$ mm.
Sucker disc: 1,5 mm lang.
Suckers: 106.
Width of head: 0,21 mm.
Diam. of disc suckers: $0{,}08 \times 0{,}04$ mm.
Diam. of oral suckers: $0{,}1 \times 0{,}07$ mm.
Ova: $0{,}17 \times 0{,}07$ mm.
Testes: 25 to 35 — $0{,}09 \times 0{,}07$ mm.
Genital pore, armed: $0{,}1 \times 0{,}08$.
Vaginal opening: 0,03.

Locality: New York Fish Market.

Name		*M. pogoniae*	*M. carangis*	*M. archosargi*	*M. angelichthys*
Author		G.A. MacCallum	G.A. MacCallum	G.A. MacCallum	G.A. MacCallum
Length		12 to 13 mm	4,4 mm	8,0 mm	4,4 mm
Breadth		1,25 mm	0,8 mm	0,8 mm	0,3 mm
Diam. mouth Sucker		0,2 × 0,13 mm	0,04 mm	0.1 × 0,07 mm bilocular	0,05 mm bilocular
Caudal disc	Length and Form	4,5 × 2,5 mm Triangular	0,9 × 0,6 Hastate	1,5 mm long Triangular	1,0 × 0,4 mm wide
	Number of Suckers	108	48	106	58
	Diameter of Suckers	0,2 × 0,15 mm	0,05 × 0,07 mm 0,04 × 0,03 mm	0,08 × 0,04 mm	0,03 × 0,02 mm
Testes		75 mm 0,1 × 0,15 mm	60 mm 0,07 mm	25 to 30 mm 0,09 × 0,07 mm	26 mm 0,06 mm
Vaginal Aperture		Median dorsal 0,04 × 0,01 No armature	Median dorsal no armature	0,03 Median dorsal	Median dorsal no armature
Cloacal Aperture		0,04 × 0,1 No armature	Separate orifices Armed-spicules Cirrus anterior	0,1 × 0,08 Conical spines and hooklets	Round sac with spicules, two separate smaller groups of spicules
Eggs		Yellow Fusiform 0,12 × 0,05 to 0,15 × 0,06	0,11 × 0,03 Yellow Fusiform Filaments both ends	0,17 × 0,07 Fusiform and with filaments	None seen
Host		*Pogonias cromis*	*Caranx crysos*	*Archosargus probatocephalus*	*Holacanthus angelichthys*

Explanation of Abbreviations.

M mouth
M. S mouth sucker
Ph pharynx
Oes esophagus
i intestine
G. P genital pore
M. G. P male genital pore
F. G. P female genital pore
Ov ovarium
Ov. d oviduct
Vit. r vitelline reservoir
Vit. d vitelline duct
Oot ootype
S. G shell gland
Ut uterus
Ova egg
Vit vitellaria
t testes
C. d caudal disc
S. C caudal disc sucker
V. d Vas deferens
Va vagina
V. O vaginal opening
S. r seminal reservoir

G. Pätz'sche Buchdr. Lippert & Co. G. m. b. H., Naumburg a. d. S.

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Notes on four Trematode Parasites of Marine Fishes.

[From the Laboratories of the United States Fish Commission, Woods Hole and the Department of Pathology, Columbia University, New York.]

By **G. A. MacCallum**, M.D., New York.

With 11 Figures.

In examining the various fishes which may be purchased in the New York fish markets or others which I have obtained through the kindness of the directors of the New York Aquarium or at the U. S. Fish Commission at Woods Hole, the following forms were found.

Acanthocotyle Bothi, n. sp.

The following sketch is of a worm found so constantly on the gills of Bothus maculatus that they may be said to be its natural habitat. Almost every fish will yield, on washing the gills, from two to twenty worms and an account of their diminutive size and the tenacity with which they cling to their attachment, no doubt only a small proportion of their actual number is ever washed off. It evidently belongs to the

genus Acanthocotyle of which Monticelli, Goto and others have written.

Monticelli[1]) in his elaborate paper on the genus Acanthocotyle describes three species, A. lobiancoi, A. oligoterus and A. elegans. Of these A. lobiancoi measures 3—6 mm in length and is provided anteriorly with two suckers and two small anterior palpi. Its circular disc is covered with radial lines of small hooklets and there is posteriorly a small round appendage. The mouth is a rather pear shaped subterminal structure. A. oligoterus is smaller, $1^1/_2$—$2^1/_2$ mm in length with a caudal disc armed with triangular hooklets and a posterior appendage with radial chitinous rods. A. elegans possesses a fringed caudal disc, again with radially arranged chitinous rods or hooklets.

Goto's[2]) species described from the examination of a single mounted specimen given to him by Professor Verrill who found it on the skin of Raja erinacea is named A. Verrilli. It has no anterior sucker but merely invaginations of the skin into which unicellular glands open. The posterior disc is covered by thirty-four radial lines of spines or hooks and there is a posterior appendage armed with filiform chitinous hooks.

From these descriptions I am forced to think that this form which I have named A. Bothi does not correspond with any of them although belonging to the same genus. A. Bothi (Fig. 1) is small, about 3 mm in length by 0.4 mm in width and of nearly equal width throughout its length. Its most notable feature is at once the large disc at its caudal end which is circular, with a diameter of 0.27 mm. It is composed of fifty-two pairs of hooks or radii covered with a musculo-membranous tissue and this is the main source of the worm's firm attachment to any sourface to which it may adhere. The radii (Fig. 2) are really hooklets of chitinous material in pairs, of about equal length throughout the circle except that at the extreme distal median part of the disc there are two longer hooks which do not extend past the outer margin of the disc but are rooted at least one-half of the distance across the free central cordate shaped space. They are conjoined at the inner margin of the circle of hooklets by a block of the same chitinous material which tends to increase their strength. This is the position of what Goto describes in his worm A. Verrilli as the round appendage and which he calls "app. post." and in which he says he counted more than twenty small hooks. In A. Bothi there are only the two reinforced long hooks in this situation. On the proximal side of the disc are two long hooks also with a square block of chitinous tissue between them. Then on each side of the circumference of the disc and between these two pairs of longer hooks there are twenty-five pairs of hooks such as shown in Fig. 1. In A. Verrilli, Goto describes seventeen on each side or half of the disc.

The head also is very different inasmuch as there are plainly visible four small suckers on each side of the central notch and apparently much of the same character as those of Nitschia elegans in miniature. The mouth is comparatively large being .22×.3 in diameter. The anterior suckers are four on each side, the two outer ones measuring .05, being larger than the middle ones, while the inner ones measure .04 mm. The worm, in making use of these suckers, is able to progress

1) Il genere Acanthocotyle. (Arch. de Parasit. I. 2.)

2) Exotic ectoparasitic Trematodes. (Journ. Sc. Coll. Imp. Univ. Tokio. Vol. 12. 1899. p. 4.)

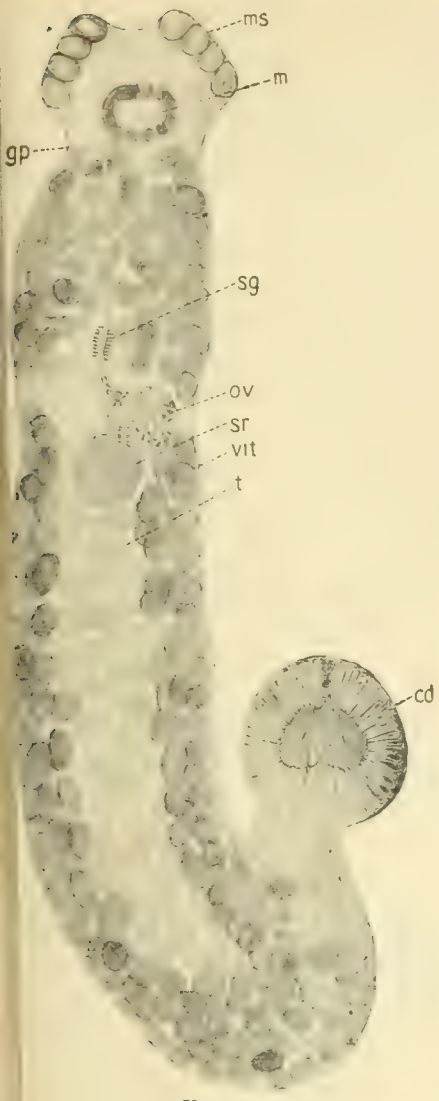

Fig. 1.

Fig. 2.

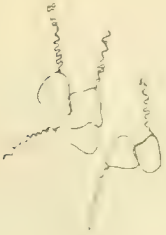

Fig. 3.

Fig. 4.

Fig. 1. Acanthocotyle Bothi showing internal structure.

Fig. 2. Acanthocotyle Bothi showing one of the radiate chitinous structures of the caudal disc.

Fig. 3. Acanthocotyle Bothi showing five eggs.

Fig. 4. Distomum trachinoti.

Explanation of Abbreviations in Figures. *m.* Mouth. *m.s.* Mouth sucker. *ph.* Pharynx. *oes.* Oesophagus. *i.* Intestine. *g.p.* Genital pore. *ov.* Ovarium. *ov.d.* Oviduct. *vit.r.* Vitelline reservoir. *vit.d.* Vitelline duct. *oot.* Ootype. *s.g.* Shell gland. *ut.* Uterus. *ova* Eggs. *vit.* Vitellaria. *t.* Testes. *c.d.* Caudal disc. *c.s.* Caudal disc sucker. *v.d.* Vas deferens. *va.* Vagina. *v.o.* Vaginal opening. *s.r.* Seminal Reservoir. *L.c.* Laurer's Canal.

from one place to another — stretching out its neck and whole body and spreading out its anterior suckers like a fan, it attaches them and lets loose with its caudal disc and draws it into proximity to the anterior end. In fact it was seen to stand with its anterior end attached to the glass while the posterior end waved about, although the natural position of the worm is upright with posterior disc attached and the anterior end waving to and fro or feeling its way about the bottom of the vessel as trematodes usually do.

The esophagus is short, and after bifurcating the intestines, immediately disappear behind the vitellaria to terminate near the caudal end. The ovary is a round body about .10 mm in diameter and is situated near the junction of the anterior with the posterior two-thirds of the body. There are about thirty-seven testes and the vas deferens proceeds forward along the right side in various convolutions ending in the common atrium with the terminus of the uterus on the right side of the neck near the head.

The vagina could not be made out but is presumably on the dorsal surface of the worm. The vitellaria are very plentiful and extend from the disc on each side to the neck.

The ovary gives off the oviduct from its anterior surface and is shortly joined by the tube from the spermatheca which is situated just behind the ovary. The oviduct is next joined by the duct from the left vitellarium and terminates in the beginning of the uterus which is surrounded by the shell gland.

I was fortunate enough while watching a number of these worms alive, to see the process of egg laying. The ova are triangular in shape, flattened and of a brown or coffee color and each has a filament attached to one angle (Fig. 3). They are apparently always laid in batches of five in quick succession or streaming at it were, and are attached to the gills as soon as laid. This was distinctly observed several times. The eggs are about .04 mm on each side of the triangle but quite flat.

Measurements.

Length: 3 × 1 mm in width.
Diam. of mouth suckers: .04—.05.
Diam. of circular sucker disc: .27.
Width of hook margin around disc showing length of hooks: .04.
Length of long hooks on distal margin of disc: .1.
Length of long hooks on proximal margin of disc: .1.
Testes: 38 in number.
Diam.: .04 to .06.
Ova: .05 × .05 triangular.
Ovary: .10 × .11.
Spermatheca: .1.
Head across: .1.
Mouth across: .22 × .2.
Oral suckers: .04 × .05.
Vaginal aperture not seen, probably dorsal.
Cloaca: on side of neck.
Host: Bothus Maculatus.
Locality: Woods Hoole, 1912, U. S. Fish Commission's Laboratory.

Distomum trachinoti, n. sp.

The worm was found in the intestine of Trachinotus Carolinus in moderate numbers. It is not large being 5.4 mm long by .8 mm to 1 mm wide and of almost uniform width throughout, except at the caudal end posterior to the ventral sucker where it becomes very much

narrower (Fig. 4). It is in this portion of the body that the testes, ovary and shell gland are situated and here too Laurer's Canal is found opening on the dorsal surface of this terminal portion.

When dead the worm is found very much curved towards the ventral surface. The skin is unarmed but is rather generally thrown into rugae. The anterior end is bluntly rounded and the anterior sucker is subterminal and about one-third of the diameter of the ventral sucker. Behind the anterior sucker is the pharynx which is large and muscular. The intestine divides almost at once and proceeds along each side almost all the way to the caudal end.

The genital pore is but little posterior to the bifurcation of the intestines and opens on the ventral surface. The body is more or less occupied with coils of the uterus filled with ova. The vitellaria are not very plentiful and are apparently confined to the sides along the middle portion of the body. The ventral sucker is a rather unusual structure beeing situated at the end of a long loose and wide semitransparent pedicle which allows of very free motion.

The ovary is small and globular, not so large as either of the two testes, behind which, it is situated near the extreme caudal end.

The uterus extends from near the ovary to the genital pore in very voluminous coils and behind these or through them the vas deferens is hidden on its way to the cirrus sac which is large. The cirrus is blunt, pointed and is supplied with a well developed prostatic portion and is situated in the atrium anterior to the outlet of the uterus.

Dr. Linton evidently has seen this worm on one occasion in Prionotus Carolinus and also in Merluccius bilinearis. He named it D. vitellosum but evidently on account of its variation of form and structure from that worm it must be of a different species. I, too, have found it once in Roccus lineatus, but Trachinotus Carolinus seems to be its natural or main habitat.

Dimensions.

Length: 5.40 mm.
Width: .1 to .80.
Length of caudal end: .80.
Diameter of anterior sucker: .49 × .45.
„ „ ventral sucker: .76 × .75.
„ „ pharynx: .25 × .20.
„ „ ovary: .25 × .20.
„ „ testes: .33 × .21.
„ „ ova: .09 × .03.
Habitat: Intestines of Trachinotus Carolinus.
Locality: Fish obtained off coast of North Carolina, U. S.

Pŏdocotyle morone, nov. gen. n. sp.

This form which seems to be confined to the gills of Morone Americana occurs in no great numbers there. At least one can wash very few of them off the gills but this may be due to their firm attachment by means of the six powerful suckers.

The worm (Fig. 5) is 6 mm in length by .6 in width and is almost of uniform width throughout, the head and tail being rounded as bluntly as possible. At about one-quarter the length of the body from the caudal end there are given off six pedicelled suckers which are relatively long beeing 4 mm in length by .15 in width. Each pedicle is surmounted by a sucker which is strengthened by a peculiar chitinous formation as shown in Fig. 7. The sucker is a little larger in diameter

Fig. 6.

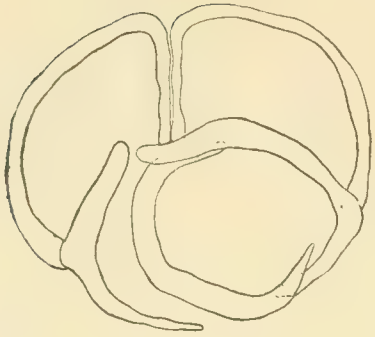

Fig. 7.

Fig. 8.

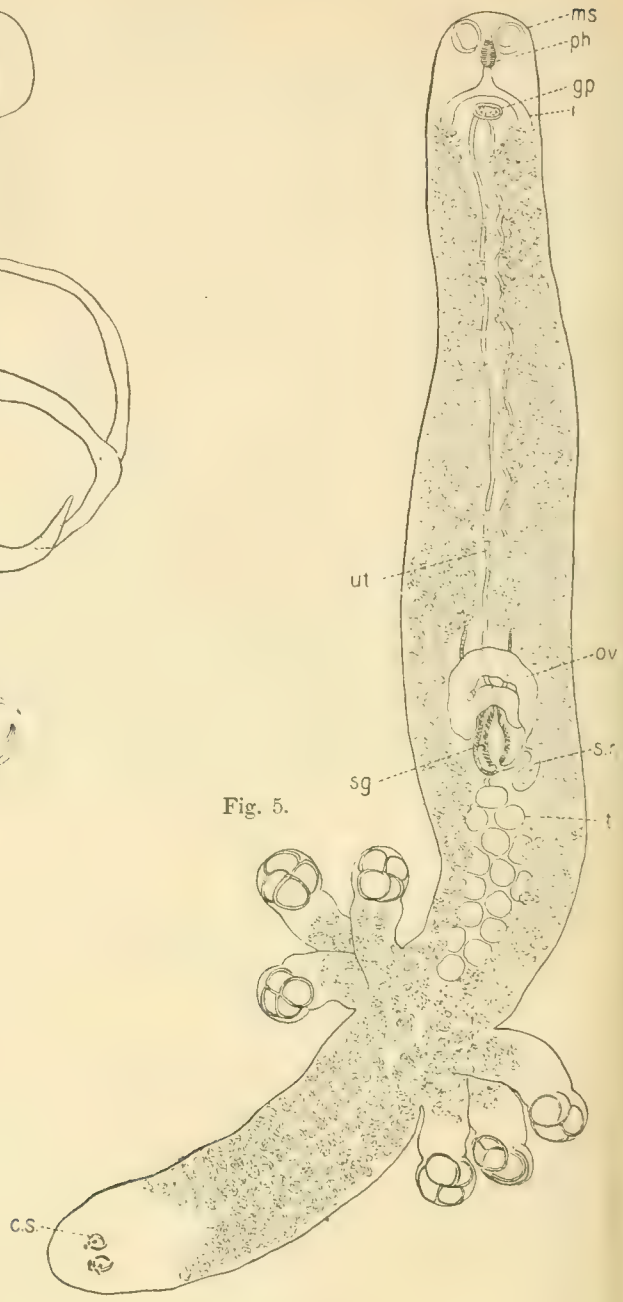

Fig. 5.

Fig. 5. Podocotyle morone nov. gen. nov. sp.
Fig. 6. Podocotyle morone showing armature of genital pore.
Fig. 7. Podocotyle morone showing chitinous skeleton of one of the lateral suckers.
Fig. 8. Podocotyle morone showing chitinous skeleton of caudal suckers.

than the footstalk on which it is placed. In life the worm is very active and the suckers are constantly protruding and opening and then retracting and closing alternately. At or near the caudal extremity are two more suckers but these are smaller and of a different chitinous structure as shown in Fig. 8. The covering membrane of all the suckers seems also to be reinforced either by chitinous filaments or muscular striae regularly arranged across the space between the larger chitinous structures.

At the anterior end the mouth is terminally situated and on each side is the ordinary oval sucker. They are not supplied with any armature but are provided with a marginal fold or lip of mucous membrane. Between the suckers is seen the pharynx which seems to have a short prepharynx in its communication with the mouth and at the junction of this with the pharynx are seen a surrounding series of nodules, six in number. The pharynx then widens out into a sac-like structure well supplied with muscular striae. Behind this the esophagus extends almost down to the genital opening before it divides into the intestinal rami which disappear behind the plentiful vitellaria.

The genital pore is seen plainly in the center of the body (Fig. 5 and 6) and is armed not very plentifully with good sized hooklets. It measures .31×.18 mm and the hooklets measure .05 mm. The vitellarium is exceedingly plentiful throughout almost the whole body, extending from opposite the genital pore almost to the caudal end. It is so plentiful that it is only with difficulty, if at all, that some of the important organs are made out, masses are seen even in the pedicles of the suckers. It is impossible to see the vaginal opening and the vas deferens which is large can only be seen at intervals between the lobules of vitellaria making its way to the genital pore and the uterus can not be made out in the anterior part of the worm at all.

The ovary stands out prominently as a tubular structure doubled on itself and placed across the body at or about its middle (Fig. 9). The mature ova are seen at the left side as is also the oviduct leading from this portion of the ovary caudal towards the plainly visible entrance to the shell gland. Shortly after leaving the ovary the duct from the seminal reservoir joins the oviduct and still further on the duct of the vitelline reservoir is plainly seen to join the common duct just before it merges into the beginning of the shell gland, which appears as surrounding a considerable portion of the beginning of the uterus. Although a fully developed ovum was seen at the entrance of the shell gland, no developed egg was present so that with the material at hand it cannot be described.

The testes are fifteen in number, of good size, but they are almost completely covered in by the vitellaria. Fig. 9, diagram of genitalia.

Habitat: Gills of Morone americana.
Locality: New York Fish Markets.
December 14th 1912.

Measurements.

Length: 6×.6 mm.
Length of caudal and posterior suckers: 1.4 mm.
Length of pedicle of suckers: .4 mm.
Diam. of suckers on pedicles: .17 to .19 mm.
Diam. of caudal suckers: .07×.05 mm.
Diam. of genital pore: .05×.06 mm.
Size of hooklets at genital pore: .01 mm.

Testes: (15) .07 × .08 mm.
No eggs.
Width of head: .25.
Oral suckers: .11 × .09.

Distomum carangis, n. sp.

On July 17, 1912, while examining a specimen of "Yellow Crevalle" Caranx crysos (Mitchell) four quite large trematodes were found together at the terminal end of the rectum. They were cream colored and about 13 mm in length by 4 mm in width (Fig. 10). Alive when found, they were sluggish in their movements but occasionally the anterior sucker would project from the center of the almost square end as the terminus of a long narrow neck, then again be withdrawn and a roundish head would be formed where the neck had been. The

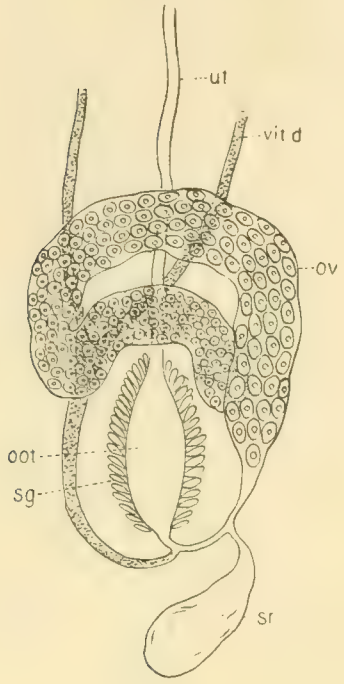

Fig. 9.

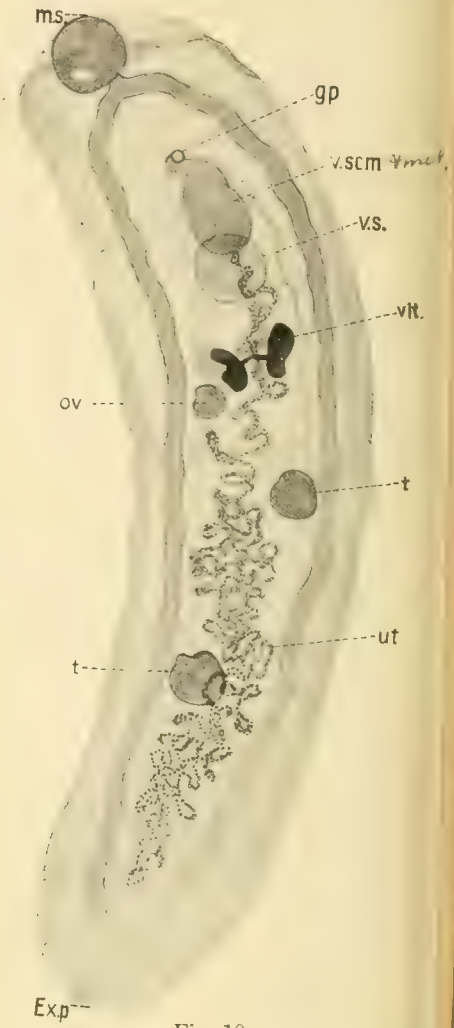

Fig. 10.

Fig. 9. Female genitalia.
Fig. 10. Distomum carangis.

body is flat and quadrilateral in shape but thicker down the center than at the sides and ends giving the impression that the body proper is surrounded by thin membranous portion or margin. On pressure between coverslips this proved to be true. for this marginal portion is quite transparent as compared with the rest of the body and is folded in all around the edge. showing that in life the ventral surface is concave since the margin could not be flattened out. The anterior or oral sucker is subterminal and comparatively small but the ventral sucker is still smaller as will be seen by the measurements given at the end of this description. The measurements of the worm are those of the body exclusive of the thin membrane or fringe surrounding it; if measured from the extreme margin of the fringe they would be much greater.

The esophagus is exceedingly short. the rami of the intestines beginning almost at the sucker and extending to near the caudal extremity, and each terminating in a cul de sac.

The genital pore is situated in the center of the body a short distance posterior to the bifurcation of the esophagus and near its angle between the rami of the intestines.

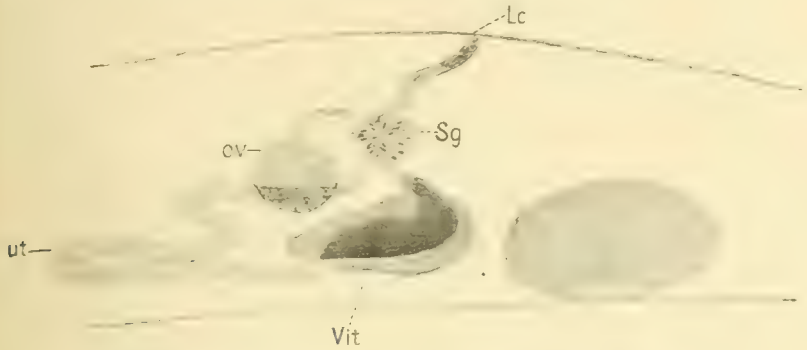

Fig. 11. **Distomum carangis showing female genitalia.**

The female genitalia: Almost immediately posterior to the ventral sucker (Fig. 11) is the vitellarium which in this case consists of two irregularly shaped lobes joined by an isthmus of the same glandular structure. The ovary is situated to the right and posterior and very near to the vitellarium. It is rather small and it is near this location that the uterus arises. since the oviduct here receives the duct from the vitelline gland. and passing through the shell gland becomes a lang coiled tube filled with ova. These coils extend between the testes to nearly the posterior extremity of the body and then passing forward to the genital pore terminate in a large sac-like metraterm which is pyriform in shape. and this in each instance examined was filled with eggs and terminated in a small mouth in a cloaca posterior to the cirrus. Very near the shell gland. as shown in serial sections. Laurer's canal joins the female genital tract from the surface dorsally. No seminal reservoir could be seen.

The male genitalia: Consist of two testes. one situated on the left side of the body and almost exactly midway in its extent lengthwise, the other being placed on the right side of the body and about one-third of its length from the caudal end. The vasa deferentia are peculiar in

that one from each testis proceeds directly and independently to the base of the cirrus sac which they pierce on opposite sides. The vasa deferentia after entering the sac unite to form the cirrus which proceeds to the genital pore where it terminates in an atrium and anterior to the outlet of the uterus. The cirrus sac is not large and is situated directly anterior to the genital pore.

The excretory system, on account of the transparency of the worm, can be readily traced to its large outlet at the caudal extremity.

Dimensions:

Length 13 × 4 mm.
Oral suckers 1.5 × .8 mm.
Ventral sucker .9 × .7 mm.
Ova small and spherical .02 × .02 mm.
Vitellaria in two masses .52 × .37 and .47 × .27 mm.
Ovary .34 × .35 mm.
Testis (left) .45 × .55 mm.
Testis (right) .80 × .57 mm.

Habitat: Rectum of "Yellow Crevalle" Caranx Crysos (Mitchell)
Loc. Woods, Hole, Mass. U.S.A.

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CILIATED CYSTS OF THE ŒSOPHAGUS

ALWIN M. PAPPENHEIMER, M.D.

The first cyst of the œsophagus described in this report was an unsuspected finding at the autopsy of a female infant aged 8 weeks, which showed no other lesions of note. Since it had been observed that the baby took its feedings with difficulty, it is probable that the cyst, which caused a distinct narrowing of the œsophageal lumen, was at least in part responsible for the infant's death.

The cyst measured 18 x 11 x 7 mm. and was situated 4.5 cm. above the cardia on the posterior wall of the œsophagus. The overlying epithelium was thinned out and translucent. The cyst contents were thin and colorless. No communication existed either with the trachea or œsophageal lumen, into which the cyst bulged.

Microscopically, the cyst is lined with epithelium which on closer study presents some interesting features. It is from two to four or five cells deep. The lowermost cells rest upon an indistinct basal membrane, and are characterized by the possession of small, slightly irregular, deeply staining nuclei. The cells superficial to this layer are of several types. One may distinguish cells with a cuticular border and very distinct long cilia. The greater portion of the cyst cavity is lined with cells of this type. Scattered amongst these, and occasionally opening into the cavity of the cyst, are typical goblet cells, the nucleus of which is compressed to a crescent lying at the base of the cell. In addition, there are a few larger rounded cells with clear cytoplasm and oval vesicular nucleus. Some of these are ciliated, others not; in either case they are readily distinguished because of their large size and clear protoplasm, from adjoining cells.

The epithelial lining of the cyst rests upon a bed of loose fibrillar connective tissue, about which are well-developed bands of smooth muscle fibers. In one section, there is found amongst these, a cross section of a tubular gland lined with a double row of non-ciliated epithelium. In the absence of serial sections, it

was not determined whether this communicated with the cyst cavity.

No other structures were found in the sections examined. The absence of plates of cartilage, mucous glands, lymphoid follicles and striated muscle fibers should be emphasized.

The œsophageal epithelium overlying the cyst is somewhat thinned as compared with that at a distance, but is not otherwise abnormal.

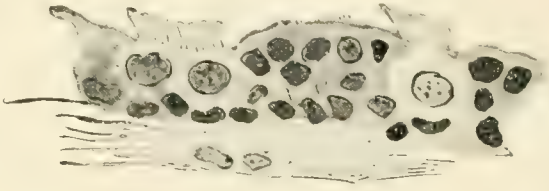

FIG. 1.

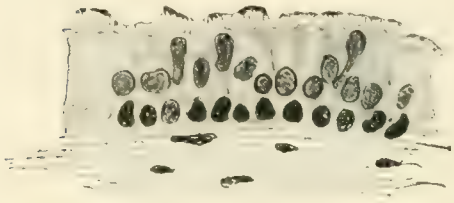

FIG. 2.

In the second case, the cysts were microscopic, and were discovered by Prof. MacCallum in a section of œsophagus taken from a small area, the gross appearance of which is described as follows: "in the upper part of the œsophagus, at the level of the larynx, there is a small ulcerated area about 5 mm. in diameter with slightly raised pearly white borders. Otherwise the œsophagus is normal."

The tissue was taken from a male, 24 years of age, who died of cardiac valvular disease.

The section shows a small plaque of mucous membrane of a character wholly different from that of the adjacent squamous epithelium. It resembles gastric or duodenal mucosa, being composed of high cylindrical epithelium lining irregular tubules or crypts which extend down into the submucosa. The latter is

densely infiltrated with lymphoid cells which in two places are aggregated in the form of follicles. The surface epithelium is largely desquamated. At the margin of the plaque, there is a nest of mucous glands, resembling Brunner's glands in the duodenum. The adjoining stratified epithelium at the edge of the plaque stops abruptly and there is no transition. It is twice as thick as the normal squamous epithelium at a distance from the plaque, and is penetrated by wandering lymphoid cells.

Three small cysts are found in the sections. They lie not beneath the patch of mucous membrane but to either side of it in the connective tissue of the œsophageal wall. They are of relatively simple structure. The somewhat irregular cavities are lined with a double row of epithelium, the inner cells of which are in most places distinctly ciliated. The basal row of cells contains darker, more spherical nuclei. There is no distinct basement membrane. The cysts are surrounded by a loose connective tissue in which are many lymphoid cells. They are scattered and not in the form of follicles. There is no proper muscular coat to the cysts, as in the first case.

Reported cases of ciliated cysts situated in or adjacent to the œsophagus are not numerous. I have been able to find reference to but fourteen cases, a brief summary of which follows:

v. Wyss: *Virch. Arch.*, 1870, li, 143.

Adult. Cyst, size of an apple, situated 1½ inches above the cardia on the posterior wall of the œsophagus. The cyst was filled with cloudy fluid. The epithelial lining was lost. No communication with the œsophagus.

Zahn: *Virch. Arch.*, 1896, cxliii, 179.

Adult. Cyst 26 x 18 mm. lying in the posterior œsophageal wall at junction of œsophagus and stomach. The cyst was lined with ciliated epithelium which was in part stratified. There was a well-developed muscular coat, in which were canals and glands. There was also a lymphoid infiltration, the cells in places being massed in the form of follicles. The derivation of the cyst is attributed by Zahn to a dislocation of œsophageal epithelium during early fetal life.

Rau, F.: *Virch. Arch.*, 1898, cliii.

Cyst, 4 cm. in diameter, located at the transition of cardia and œsophagus, on the right posterior wall. The cyst was lined with ciliated epithelium of relatively simple structure.

TRESPE: *Arb. aus d. path.-anat. Abteil. des hygien. Inst. zu Posen von Lubarsch,* Wiesbaden, 1901.

Male infant, less than two months of age. Bilocular cyst, size of pigeon's egg, situated between thoracic aorta and œsophagus 4 cm. above the cardia. The cyst did not communicate with the lumen of the œsophagus, but lay partly within the œsophageal musculature, on the posterior wall. Histologically, the cyst was lined with ciliated epithelium, but contained in its wall scattered plates or "rings" of hyaline cartilage.

From the resemblance of the cyst wall to trachea or bronchus Trespe concludes that the cyst arose through a dislocation (Abschnürrung) of tissue derived from the fetal fore-gut, before or during its differentiation into respiratory and œsophageal portions.

KRAUS: *Nothnagel's Hdbch. d. Spez. Path., etc., Erkrankungen d. Mundhohle u. d. Speiserohre,* 1902.

Male, 25 years of age. Cyst in anterior wall of œsophagus, at level of tracheal bifurcation, 6 x 3 cm. in size. The cyst was filled with brownish fluid and lined with ciliated epithelium.

v. SKOPNIK: *Inaugural Diss.,* Munich, 1907.

The cyst was found in a two-year old boy, dying of diphtheria. It measured 13 mm. in diameter, and was situated in the lower part of the œsophagus, towards the right and anteriorly. It contained cloudy, viscid fluid, and was lined with rather low ciliated epithelium.

DURCK: *Muench. Med. Woch.,* 1907, liv, 2165.

Male child of 2 years dying of diphtheria. In the lower part of the œsophagus to the right of the median line, there was found a cyst the size of a hazel nut, with translucent walls. Sections showed a continuous lining of cylindrical ciliated epithelium surrounded by the double layer of smooth muscle fibers.

(Probably the same case as v. Skopnik.)

MOHR: *Ziegler's Beiträge,* 1909, xlv, 325.

Case I.—Woman, aged 81 years. Two-chambered cyst, each cavity about the size of a walnut. The lower pole of the mass, which bulged into the pleural cavities, lay about 3 cm. above the cardia. The cyst was lined for the most part by a single layer of high cylindrical ciliated cells, beneath which there was a row of somewhat more irregularly disposed globular cells with pale protoplasm. In one place, there was found in the wall of the cyst, a plate of cartilage, surrounded by connective tissue.

Case II.—Male, 28 years old, dying of pulmonary tuberculosis. Walnut-sized cyst, 5 cm. above the cardia, on the left side. The cyst was lined with ciliated epithelium, thrown up into papillary folds. Outside of the connective tissue tunica propria which was in places infiltrated with lymphoid cells, were bundles of smooth muscle fibers.

Case III.—Male, aged 65 years, dying of arterio-sclerosis and encephalomalacia. One cm. above the trachea, on the left and posterior wall of the œsophagus, a cyst measuring 3 x 2 cm., bulging into the lumen of the œsopha-

gus, and covered by smooth mucous membrane. The cyst was lined by a simple layer of cylindrical ciliated epithelium, thrown up in places into papillæ.

STAEHELIN-BURCKHARDT: *Arch. f. Verdauungskr.*, 1909, xv, 584.

Female, 9 months old, operated for spina bifida. Cyst measuring 5.8 x 4.5 x 2.5 cm. just above diaphragm. The surface covered by pleura save where the cyst was in contact with the œsophagus. The epithelial lining was of variable thickness, in places consisting of a row of simple cuboidal cells, in other portions ciliated, in still other regions presenting definitely the character of gastric mucosa, with glandular crypts. In the sub-mucous connective tissue, follicle-like aggregations of lymphoid cells. There was a distinct muscularis mucosæ, and muscular layer.

The origin of the cyst is attributed to an early misplacement of œsophageal epithelium, including the anlage of cardiac glands. The latter, in their further differentiation, gave rise to the gastric mucosa.

KERN: *Virch. Arch.*, 1910, cci, 135.

Male child of 7 months. On dissecting œsophagus from trachea, a pea-sized cyst was discovered, lying in the tunica adventitia of the œsophagus at the level of the upper pole of the thyroid. The cyst was divided into three chambers, separated from each other by connective tissue septa. The cyst was lined by a single layer of ciliated cells. The wall was composed of connective tissue in which were found plates of hyaline cartilage, mucous glands and ducts lined with cuboidal epithelium. Smooth muscle fibers were not found.

Because of the presence of cartilage and mucous glands, Kern interprets the cyst as an evagination and subsequent constriction of tissue derived from the trachea, and not from the œsophagus.

STOEBER: *Ziegler's Beiträge*, 1912, lii, 512.

Infant of 3 months, upon which had been performed a tracheotomy and subsequent thymectomy for supposed respiratory obstruction. At the autopsy, there was found a pigeon-egg sized tumor on the anterior and lateral wall of the œsophagus, extending from about 1 cm. below the lower pole of the thyroid to within 2 cm. of the bifurcation of the trachea. The cyst measured 5 x 3 cm. It lay directly beneath the mucosa of the œsophagus, and did not communicate with the lumen of the œsophagus or trachea. On section, it was unilocular.

Microscopically, the cyst was lined by two to five layers of epithelial cells, in part ciliated. There were occasionally ducts lined wtih cubical epithelium in the cyst wall. Striated muscle fibers were found in places amongst the smooth muscle bundles of the cyst wall. On closer study of the lining epithelium of the cyst, various types of cells were found corresponding to those described by Schridde as occurring in the fetal œsophagus. Amongst these, was a single group of very high cylindrical cells, probably representing an island of gastric mucosa.

COESFELD: *Inaug. Diss.*, Kiel, 1891 (not accessible).

From the cases described, it will be seen that there is considerable variation in the size of the cysts. The largest is that reported by Kraus which measured 6 cm. in length. The cysts vary further, in their location, several having been situated at the cardia, others at various points as high up as the thyroid cartilage. The sites of predilection seem to be either at the junction of œsophagus and cardia, or in the neighborhood of the tracheal bifurcation. They may be found either in the anterior or the posterior wall of the œsophagus. In none of the cases was there a communication with the lumen of the gut.

As regards the finer structure of the cysts, there appears to be considerable uniformity in the character of the lining epithelium. This is always, at least in part, ciliated; it may be either in a single layer, or more frequently, there are two or more layers of cells. In addition to the ciliated cells, cells of various types such as occur in the normal œsophagus during fetal life, may be found. In the article of Stoeber will be found a detailed and well-illustrated study of the different types of cells found in the lining epithelium. With the exception of high cylindrical gland cells of the gastric type, all the forms depicted by Stoeber were found in the writer's case.

The epithelium rests upon a loose connective tissue in which collections of lymphoid cells may be found. A definite basement membrane is not noted in any of the cases. The cysts are almost always surrounded by bands of smooth muscle fibers; only in Stoeber's case is there mention of striated fibers.

Plates of cartilage are described in the cases of Mohr, Trespe and Kern. Whereas the last of these writers for this reason believes the cysts to be derived directly from the trachea, Trespe and Mohr are inclined to believe that the presence of these tracheal constituents indicates merely a very early separation of the tissue from the œsophagus, before or during the time of the earliest development of the respiratory bud. Bert and Fischer have gone a step further, and interpret all ciliated œsophageal cysts, whether or not containing cartilage and mucous glands in their wall, as accessory lung anlages. They thus consider them

comparable to the accessory lungs found in the pleural cavities, and occasionally below the diaphragm. They maintain that the ciliated epithelium and the plates of cartilage may be taken as indicating the tendency of œsophageal tissue, when separated from its connections, to undergo differentiation along the line of development of the respiratory system. Stoeber, on the other hand, sees in the ciliated character of the epithelium merely a persistence of the embryonic type of œsophageal epithelium, and the other types of cells as comparable also to the varied forms into which the basal layer is differentiated during fetal life.

It is quite possible that both views are correct; that in the simpler cysts, we are dealing merely with a misplacement of fetal œsophageal tissue, whereas in those cysts the structure of which is complicated by the presence of cartilage and mucous glands, we have in addition to a displacement of œsophageal tissue, a tendency toward the development of a rudimentary lung anlage. The fact that these cartilage-containing cysts are occasionally given off from the posterior-wall of the œsophagus, does not negative this interpretation, since we are dealing with aberrant, anomalous structures.

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Bert u. Fischer: Uber Nebenlungen und versprengte Lungenkeime. *Frankf. Ztschr. f. Path.*, 1911, vi, 27.

OBSERVATIONS ON A SPONTANEOUS TYPHOID-LIKE EPIDEMIC AMONG WHITE RATS.*

ALWIN M. PAPPENHEIMER AND HASSOW VON WEDEL.

(From the Departments of Pathology and Bacteriology of the College of Physicians and Surgeons, Columbia University, New York City.)

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I. INTRODUCTION.

During the past winter, many of the albino rats used in this laboratory died of an acute infectious disease. Among the rats which became infected were five litters, the majority of which had been thymectomized when from 10 days to 2 weeks old, and which had been kept under close observation for several months. As these rats showed symptoms of illness, they were killed together with control, unoperated animals of the same litters, and a routine histological examination made of the principal organs. The lesions found proved to be so striking and to resemble in so many respects the lesions of human typhoid fever, that it seemed desirable to isolate the infecting organism and to study the disease experimentally.[1]

Interest in epidemics among rats and mice dates back to the first publication of Löffler[2] in 1892. Löffler isolated from spontaneously infected laboratory mice a motile, gram-negative bacillus of the typhoid-colon group to which he gave the name *B. typhi murium*. This bacillus was pathogenic for field mice, but not regularly so for house mice; grey rats could not be infected. The following year Löffler used cultures of this organism to combat a plague of field mice which were ravaging the crops in

* Received for publication August 19, 1913.

[1] A preliminary report of our work was presented at the meeting of the New York Pathological Society in May.

[2] *Centralbl. f. Bakteriol.*, 1892, 11, p. 129; 1893, 13, p. 647.

Thessaly, and his successful experiences inaugurated the use of bacteria of this group for the wholesale destruction of rodents.

Danysz[1] in 1900 isolated from an epidemic of field mice in Charny-en-Seine a similar gram-negative bacillus which at first exhibited marked virulence for rats. The Danysz virus has been repeatedly studied, both as to its bacteriological relationships and as to its practical applicability to the destruction of rats.

Issatschenko,[2] Tartakowsky,[3] Schilling,[4] Trautmann,[5] and Schern[6] have described spontaneous epidemics among laboratory rats, in all of which bacilli of the typhoid-colon group have been isolated. There is further an abundant literature dealing with the interrelationships of the different strains of rat and mouse viruses, and their identity with one or another type of paratyphoid, enteritis, or hog cholera bacillus. We may refer here to the papers of Bahr,[7] Raebiger and Grosse,[8] Bainbridge,[9] Rosenow,[10] Kutscher and Meinicke,[11] Xylander,[12] Mereschowsky,[13] Bongert,[14] and Steffenhagen,[15] and shall have occasion to cite some of these workers in discussing the identity of the bacillus isolated by us.

Interest in the study of these organisms has thus centered largely upon their practical use in the extermination of rats and mice, and to this end cultures prepared by various hygienic institutes and commercial firms have been used on a large scale. Furthermore, the close relationship of the "Rattenschadlinge" to paratyphus and meat-poisoning bacilli, has suggested that their indiscriminate use might give rise to human infections, and this possibility appears to have been established by the experiences of Handson, Williams, and Klein,[16] Mayer,[17] Trommsdorf,[18] Shibayama[19] and Fleischanderl.[20]

In contrast to the abundant literature dealing with the bacteriologic and hygienic aspects of the disease are the relatively scant references to the pathological features. The gross changes, it is true, have been repeatedly described, and with considerable uniformity save as regards the intestinal lesions. On the other hand, so far as we are aware, a careful histological study has not been published. Mallory and Ordway in 1909[21] first called attention to the resemblance of the lesions produced by the injection of the Danysz bacillus in rats to the lesions of human typhoid, and demonstrated illustrative preparations at the meeting of the American Association of Pathologists and Bacteriologists. Beyond a brief abstract, no complete report of their work has appeared. During the progress of our study, there appeared a paper by Ordway, Kellert, and

[1] *Ann. d l'Inst Pasteur*, 1900, 14, p. 193

[2] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1898, 23, p. 873; 1902, 31, p. 26.

[3] Ref. in *Baumgarten's Jahresber.*, 1902, 18, p. 597.

[4] *Arb. a. d. k. Gsndhtsamte*, 1902, 18, p. 108.

[5] *Ztschr. f. Hyg.*, 1906, 54, p. 104.

[6] *Arb. a. d. k. Gsndhtsamte*, 1909, 30, p. 575.

[7] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1905, 39, p. 263.

[8] *Ibid.*, 1910, 54, p. 231.

[9] *Jour Path. and Bacteriol*, 1909, 13, p. 443.

[10] *Bull. No. 5, Hyg. Lab., U.S. Marine Hosp. Serv.*, 1901.

[11] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1905, 52, p. 301.

[12] *Ztschr. f. Fleisch- u. Milchhyg.*, 1908, 18, p. 246.

[13] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1895, 17, p. 742.

[14] Kolle u. Wassermann, *Handbuch d. Path. Mikroorg.*, Ergzheft, 1903, 3, p. 742.

[15] *Arb. a. d. k. Gsndhtsamte*, 1911, 36, p. 198.

[16] *Brit. Med. Jour.*, 1908, 2, p. 1547.

[17] *München. med. Wchnschr.*, 1905, 47, p. 2261.

[18] *Arch. f. Hyg.*, 1906, 55, p. 279.

[19] *München. med. Wchnschr.*, 1907, 64, p. 979.

[20] *Ibid.*, 1909, 56, p. 392.

[21] *Jour. Am. Med. Assn.*, 1909, 52, p. 1455.

Huested[1] on "A Typhoid-like Disease in Rabbits, Produced by the Subcutaneous Inoculation of a Strain of *B. suipesticus*." The lesions noted by them in this experimental disease are in many respects identical with those obtained by infecting rabbits with the organism isolated by us.

II. SYMPTOMS OF THE DISEASE.

We have, unfortunately, no data as to the percentage of infections or mortality. Of 11 young rats amply exposed to direct contact infection, 8, or 72 per cent, showed at autopsy characteristic lesions of the disease; the remaining 4 rats evidently escaped infection, or were so mildly infected that no traces of the disease could be found. The deaths in the stock cages during the winter months were very numerous, but during the late spring, no rats became spontaneously infected, the epidemic evidently having spent itself. The duration of the disease, as it occurred spontaneously, could not be accurately determined, but appeared to vary within wide limits. In several litters which had been under observation for some time and which were being weighed daily, the duration of the disease, as measured by the initial loss of weight, was 3, 4, 7, and 12 days respectively. These data are only approximate, as the animals were killed when moribund. Moreover, in two rats, characteristic lesions were found altho there had been no previous loss of weight.

In animals experimentally infected and allowed to die, the duration of the illness varied with the mode of infection. Two rats after intraperitoneal injection of a large dose died after two and three days respectively; after subcutaneous inoculation death occurred in six and seven days; after infection by feeding, in nine days.

Emaciation and loss of weight were very marked in most of the rats. There was regularly observed a marked anemia, as judged by the blanching of the ears and the pallor of the eye-grounds. Smears from the blood showed great numbers of normoblasts, and marked polychromatophilia and granular degeneration of the erythrocytes. The normal pink color of the eyes was often changed to a brownish or chocolate tinge by which the disease could be readily diagnosticated. This has been shown by Boycott[2] to be due to methemoglobinemia. A bloody crust about the nose and eyes was always present in the terminal stages. Diarrhea occurred in

[1] *Jour. Med. Research*, 1913, 28, p. 41 [2] *Jour. Hyg.*, 1911, 11, p. 443

some, but by no means in the majority of the cases. Many of the rats showed no intestinal disturbances, and voided normal dry globular scybalae until their death.

III. BACTERIOLOGICAL FINDINGS.

A bacteriological study was made of three rats dying of spontaneous infection during this epidemic. The cultures obtained were compared with one another morphologically and biologically to establish their identity, and were then inoculated into four rats and fed to two others to fulfill Koch's postulates for determining the etiological relationship of a microorganism to a disease. Other animals were also experimentally inoculated. The strains isolated were also compared with the following cultures:

1. *B. enteritidis* Gaertner, obtained from the collection of the American Museum of Natural History, New York City.

2. *B. typhi murium* A, also obtained from the American Museum of Natural History, is a subculture from a strain isolated by S. Miggi from the "Liverpool virus," a commercial rat poison sold in England and in this country.

3. *B. typhi murium* B, a strain from our laboratory collection.

4. Bacillus from the Rockefeller Institute, isolated during a rat epidemic by Dr. F. Haines.

5. *B. paratyphosus* B. (Schottmüller), from the laboratory collection.

6. *B. coli communis*, from the laboratory collection.

7. *B. typhosus* (James), from the laboratory collection.

8. *B. typhosus*, isolated by one of us from a patient with typhoid fever.

METHODS EMPLOYED IN THE COMPARATIVE TESTS.

Indol and nitrate cultures were examined four, six, and fifteen days after inoculation and were then kept for a few weeks. The observations on the sugar fermentation tubes were made after 48 hours. All fermentation comparisons were made with tested sugars in sugar-free broth. Four degrees of gas production were noted, namely + = trace of gas; ++ = one-fourth of closed arm full of gas; +++ = half-closed arm full of gas; ++++ = three-fourths of closed arm full of gas. Negative control tubes were used in all tests.

Agglutination tests were invariably made by the macroscopic method. Emulsions of the bacteria of approximately the same degree of opalescence were made in normal saline solution from agar cultures grown at 37° for 18 hours. The agglutination readings were taken after incubating the tubes for two hours, and again after four hours; they were then kept at room temperature, and a third reading was taken the next morning. The four-hour reading is used in the following table. In all cases the highest dilution of the serum at which agglutination was visible to the naked eye was taken as the agglutination limit of the serum. Control tubes containing only bacterial emulsion and normal saline were used in every experiment. The serum used was

monovalent and was obtained from a rabbit which had received five injections of dead, and five of live, bacteria. The injections were made daily, with four days' interval between administering the dead and the live bacteria. The bacteria used for immunization were of the strain isolated from Rat A2. Three degrees of agglutination were noted, namely, + = complete reaction; ± = partial reaction; − = negative reaction. All tests were made in duplicate.

THE INVESTIGATION OF THE RAT EPIDEMIC.

Rat A1 had profuse diarrhea, and there were crusts of blood about eyes and nose. At autopsy, there were obtained from the heart blood, liver, and spleen pure cultures of a gram-negative bacillus. This was slender and actively motile, resembling morphologically the typhoid bacillus.

Rat A2 was examined about six hours after death. From the heart blood, liver, and spleen in all plates, pure cultures were obtained of an organism identical morphologically and culturally with the gram-negative bacillus found in Rat A1.

Similarly Rat A3 was examined and pure cultures of this organism were again obtained from the heart blood, liver, and spleen. Other rats were examined during the epidemic and gram-negative bacilli were found in all, but a complete cultural identification was not made.

The bacilli were readily isolated in pure culture from all the rats examined.

MORPHOLOGICAL AND CULTURAL CHARACTERS.

The bacillus is slender, actively motile, gram-negative, and non-spore-bearing. It stains with the ordinary laboratory dyes. In the tissues of the rats the bacillus appeared much larger than in smears from the cultures and showed bipolar staining. On agar plates, the organism grew rapidly, forming moist grey-white colonies which were large and round with smooth borders. On agar slants the growth had the same grey-white moist appearance. The water of condensation was cloudy with a heavy sediment for the first few days; after standing for a week, this cleared up for the most part.

Gelatine stab-cultures showed a fungiform growth and were not liquefied in 30 days.

Broth was evenly clouded in 24 hours. Afterward a heavy

sediment and a thin pellicle formed. At the end of four days the sediment collected in large flakes at the bottom of the tube.

Nitrates were reduced to nitrites. In Dunham's peptone broth, there was slight indol production after four to six days in nearly all tests.

On potato, a scant grey-white growth was obtained that became visible only after three to four days.

Litmus milk at the end of 24 hours was acidified, at the end of 48 hours neutral, and at the end of 3 days alkalin. It was not coagulated.

B. enteritidis Gaertner and *B. typhi murium* A were compared with the organisms isolated from the rats on all the culture media just described. No marked difference was found in any of their cultural characters.

The bacilli isolated from the rats were also compared on serum water carbohydrate media and on fermentation tubes, with *B. typhi murium* A, *B. typhi murium* B, and the rat bacillus from the Rockefeller Institute. Our first series of comparisons were made in April, 1913, very soon after isolating the organisms from the rats. Fermentation tubes were then inoculated with the first subcultures of A1 and A3: A2 had been transplanted about 15 times. The organisms taken for comparison were from stock cultures. The results are as follows:

TABLE 1.

SUGAR FERMENTATION REACTIONS.

Strains	Rat B (A1)	Rat B (A2)	Rat B (A3)	*B. typhi murium* A	*B typhi murium* B	Rat Bacillus from Rockefeller Institute
Dextrose	Acid	±	Acid	+	+++	++
Maltose	+*	+	+	+	++	++
Levulose	+	+	+	++	++	++
Lactose	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin
Mannite	+	+	±	+	++	+++
Saccharose	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin
Dextrin	"	"	"	"	"	"
Galactose	+	+	+	+	++	++

* Sign + indicates gas production in fermentation tubes; the number of + signs indicates the degree of gas production.

These results were published in our preliminary paper on this subject. Since that time, about 30 daily transplantations on

glucose agar slants have been made, and we have again compared the gas production with the results tabulated:

TABLE 2.

SUGAR FERMENTATION REACTIONS

Strains	Rat B (A1)	Rat B (A2)	Rat B (A3)	*B. typhi murium* A	*B. enteritidis* Gaertner	*B. typhi murium* B	Rat Bacillus from Rockefeller Institute
Dextrose	+++	+++	+++	+++	+++	+++	+++
Maltose	+	+	+	+	+++	+++	+++
Levulose	++	++	++	++	++	++++	++++
Lactose	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin
Mannite	− − +	++++	+++	++++	++++	++++	++++
Saccharose	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin	Alkalin
Dextrin	"	"	"	"	"	"	"
Galactose	++	+++	++	++	++	++++	++++

As may be seen by comparing the two tables, all cultures showed a greater degree of gas production after growing on glucose agar for about two months. All three strains of the rat bacillus produced abundant gas in the glucose fermentation tubes. The rat bacillus from the Rockefeller Institute and *B. typhi murium* B differed from the others only in the degree of their gas production. The three strains of our rat bacillus, *B. typhi murium* A, and *B. enteritidis* Gaertner agreed in every instance except in the degree of gas production in maltose.

AGGLUTINATION REACTIONS.

The agglutinability of our three strains of organisms was compared with strains of *B. typhosus* (James), *B. typhosus* (v. W), *B. paratyphosus* B (Schottmüller), *B. coli communis*, *B. enteritidis* Gaertner, *B. typhi murium* A, *B. typhi murium* B, and the rat bacillus from the Rockefeller Institute. The results are tabulated in Table 3.

These agglutination reactions indicate that the organisms isolated from the rats differ markedly from *B. coli*, *B. paratyphus* B, *B. typhi murium* B, and the Rockefeller Institute rat bacillus. It may also be seen that the two strains of *B. typhosus* agglutinated in quite high dilutions. This curious phenomenon has also been reported by Hübener,[1] Lebram,[2] and others. They have found that

[1] *Fleischvergiftungen u. Paratyphus Infektionen*, Berlin, 1910.

[2] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1909, 44, p. 411.

TABLE 3.

AGGLUTINATION REACTIONS.

Agglutination with Serum of Rabbit Immunized with Rat Bacillus No. A2. Time of Recorded Readings: 4 Hours.

Strains	Rat B (A1)	Rat B (A2)	Rat B (A3)	*B. typhi murium* A	*B. enteritidis* Gaertner	*B. typh* (James)	*B. typh* (V. W)	*B typhi murium* B	Rat B Rockefeller Institute	*B. coli*	*B. paratyph.* B
Dilution											
1- 20	+	+	+	+	+	+	+	−	−	−	−
1- 80	+	+	+	+	+	+	+	−	−	−	−
1- 120	+	+	+	+	+	+	+	−	−	−	−
1- 200	+	+	+	+	+	+	+	−	−	−	−
1- 250	+	+	+	+	+	+	+	−	−	−	−
1- 300	+	+	+	+	+	+	+	−	−	−	−
1- 600	+	+	+	+	+	−	+	−	−	−	−
1- 1,200	+	+	+	+	+	−	−	−	−	−	−
1- 2,500	+	+	+	+	+	−	−	−	−	−	−
1- 5,000	+	+	+	+	+	−	−	−	−	−	−
1-10,000	+	+	+	+	+	−	−	−	−	−	−
1-15,000	+	+	±	+	±	−	−	−	−	−	−
Saline Control	−	−	−	−	−	−	−	−	−	−	−

the typhoid bacillus at times agglutinates almost up to the titer limit of the serum of *B. enteritidis* Gaertner.

These agglutination experiments seem to confirm the results of our culture comparisons and suggest that the organism isolated from the rats, *B. enteritidis* Gaertner, and the organism of the Liverpool virus are very closely allied.

The close relationship of the organism of the Liverpool virus and *B. enteritidis* Gaertner has previously been reported by Steffenhagen[1] who made an extensive investigation to determine this fact. He reported that they agreed in practically all respects. The work of Mühlens, Dahm and Fürst,[2] von Ermengen,[3] Schern,[4] and others suggests that both in their cultural characteristics and in their agglutination reactions the organisms isolated by them from rat epidemics agreed with the Danysz, Ratin, Issatschenko, and Gaertner bacillus.

Bainbridge[5] also reports that *B. enteritidis* Gaertner and the Danysz bacillus, which can be easily distinguished from *B. paratyphus* A, *B. paratyphus* B, *B. Aertryck*, and *B. suipesticus* by their agglutination reactions, are indistinguishable from one another

[1] *Op. cit.*

[2] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1909, 48, p. 1.

[3] Kolle u. Wassermann, *Handbuch d. Path. Mikroorg.*, 1903, 2, p. 637.

[4] *Op. cit.* [5] *Op. cit.*

and apparently, also, are merely strains of the same organism. He also reports that *B. typhi murium* has no existence as a definite organism, since different strains alleged to be *B. typhi murium* and obtained from accredited sources were found to differ greatly. His reports seem to agree with our findings.

It is therefore evident that the bacillus which we have isolated from this epidemic agrees in nearly all respects with *B. enteritidis* Gaertner and with *B. typhi murium* A which we secured from the American Museum of Natural History and which is a subculture of a strain originally isolated from the Liverpool virus, a commercial rat poison sold in England and this country.

This Liverpool virus has also been investigated by Handson, Williams, and Klein.[1] An epidemic broke out in a large business house in London. Twelve persons were taken ill with the disease. Ten days later when they were all convalescent, an investigation revealed the fact that, altho these men all ate at the business house with many other employees, only those who ate in a certain room were taken ill. In this room a bad odor was noticed, and upon removal of the floor boards, 40 dead rats were found. It was then discovered that a rat poison, the Liverpool virus, had been spread on bread and placed around the room so that the rats could get it. Cultures were made from the dead rats and from the patients, and Klein reports that the bacilli isolated agreed in every respect with each other, and with the organism found in the Liverpool virus. Blood sera of the convalescent patients agglutinated the organisms of the Liverpool virus, the organism isolated from the dead rats, and the organism isolated from the patients.

During an investigation of mouse typhoid, Mayer,[2] who was conducting the work, was taken violently ill. Upon examination of his stools mouse typhoid bacilli were isolated. His blood serum in high dilutions agglutinated the mouse typhoid bacilli. He therefore concludes that mouse typhoid bacilli can cause acute and violent illness in man.

Four separate outbreaks of gastro-enteritis, with one death, have been observed by Shibayama[3] in Japan, who shows reasonable grounds for attributing them to the careless use of a rat virus containing Löffler's *B. typhi murium*.

[1] *Op. cit.* [2] *Op. cit.* [3] *Op. cit.*

ANIMAL INOCULATIONS.

In order to establish the etiological relationship of the organism to the disease, we inoculated six rats brought into the laboratory especially for these tests from a lot that had no record of disease among them and which were kept under observation for several days.

Two rats were inoculated intraperitoneally with 0.5 c.c. of a saline suspension of the bacilli grown on agar for 24 hours. These rats died in two and three days. They developed no characteristic symptoms.

Two others were inoculated subcutaneously with the same amount of bacterial suspension; they had no diarrhea, but bloody crusts about eyes and nose formed in five days and the animals were dead in six and seven days.

The last two were fed with bread soaked in 24-hour broth culture; they developed all the characteristic symptoms and died in nine days.

At autopsy, the organisms were recovered in pure culture in all six experimental tests from the heart blood, liver, and spleen, and the characteristic lesions were present.

These results therefore clearly establish the etiological relationship of the bacilli to the disease.

We then inoculated a rabbit, a guinea-pig, and two mice, one intraperitoneally and one subcutaneously with a saline suspension of the bacilli. One c.c. of the bacterial suspension killed a rabbit in three days, 1 c.c. killed the guinea-pig in 18 hours, and one-third of a cubic centimeter killed the two mice in 18 hours.

In every case, the organisms were recovered in pure culture and in smears from the heart blood of the animals; large numbers of the bacilli were found, showing that the organism had developed, giving rise to a true infection. It was difficult to find the bacilli in smears from the heart blood of the rats dying of spontaneous infections, altho they grew in cultures. We also inoculated a rabbit with the same amount of bacterial suspension which had been given to the other animals, killed by heat, as a control for the virulent inoculated material of the cultures. The rabbit was not killed.

As yet we have not tried to infect other animals by feeding.

The majority of workers, however, have not been able to reproduce the disease in rabbits, cats, and dogs by feeding them with the bacillus isolated from epidemics in rats.

Conclusions.

The organisms isolated from the rats proved to be etiologically concerned in causing the epidemic. They were found to agree with the bacillus of the Liverpool virus and *B. enteritidis* Gaertner, both in their cultural characters and in their agglutination reactions. This suggests the probability that all these bacilli are very closely related to one another, if not strains of the same organism. It would seem, further, that the use of commercial rat viruses containing these organisms may be a menace to man.

IV. PATHOLOGICAL FINDINGS.

1. Previous Observations.

The lesions observed by Löffler[1] in the mouse epidemic described by him were enlargement of the spleen, necrotic foci in the liver, and hemorrhagic gastro enteritis. The Peyer's plaques and the mesenteric lymph-nodes were swollen and congested. Masses of bacilli were always found in the necrotic areas in the liver, but occurred also in the liver capillaries apart from the necroses.

Schilling[2] in 1902 published an account of a spontaneous epidemic among laboratory rats. The duration of the illness was only one or two days, the rats showing weakness, roughness of the hair, closing of the eyes by crusts, and in most cases diarrhea. Retardation of growth was observed in those rats which escaped the disease. Pathologically, there was found enlargement of the spleen. The stomach was normal, but the middle portion of the small intestine exhibited various grades of inflammation and edema, with swelling of the follicles. In the mild cases, the intestinal contents consisted of thin fluid, with gas bubbles; in the severe cases, of mucus admixed with blood. The lower portion of the small intestine, the cecum, and colon were unchanged. The lungs in the acute cases, showed merely petechial hemorrhages and small areas of collapse, but in the more protracted forms of the disease, the lungs were dense, infiltrated, and contained yellowish areas of necrosis. Schilling thus distinguishes two forms of the disease, one acute, with predominantly intestinal lesions, the other more chronic, with marked alterations of the lungs.

Trommsdorf[3] in 1903 gave a fairly detailed description of the lesions produced in white rats by feeding bacilli isolated from the feces of patients suffering from an epidemic of dysentery. These bacilli were agglutinated by mouse typhoid serum, and were considered by Trommsdorf to be identical with *B. typhi murium*.

The spleen in the infected mice was enormously enlarged, and usually firm. The liver was dark red and showed fatty areas, or was sprinkled with yellowish points. The kidneys were normal. The small intestine was edematous, injected, and filled

[1] *Op. cit.* [2] *Op. cit.* [3] *Op. cit.*

with fluid feces. The mucosa was sprinkled with hemorrhages. The mesenteric, inguinal, and axillary lymph-nodes were swollen and hemorrhagic. The lungs were normal. Bacilli were found in smears from liver and spleen and in cultures from all organs.

Bahr[1] in 1905 made a comparative study of the Danysz and Issatschenko viruses, of a commercial virus called "Ratin," and of *B. typhi murium*. Incidentally, he studied the lesions produced by feeding brown rats with cultures of "Ratin." The lesions noted are the same as those recorded by previous observers, save that Bahr mentions the occurrence of fibrinous peritonitis.

Trautmann[2] in 1906 described the lesions found in epidemic and sporadic infections among laboratory rats at the Hamburg Hospital, and emphasized the resemblance of the clinical picture to that of rat plague. The autopsy findings in both spontaneously and experimentally infected rats were emaciation, hemorrhagic bubos, swelling and punctiform necroses of the liver, marked enlargement of the spleen, hyperemia of the lungs, often with necrotic foci. In feeding experiments, there was swelling of the Peyer's plaques and of the mesenteric, retroperitoneal, and submaxillary lymph-nodes. No microscopic studies were made.

In 1906, there appeared also an exhaustive paper by Kutscher and Meinicke,[3] dealing with the comparative cultural and biological characters of numerous strains of *B. paratyphus* A and B, *B. enteritidis* Gaertner, and *B. typhi murium*. The pathogenicity of the various strains was tested on mice, rabbits, and guinea-pigs, but aside from the gross lesions noted by previous observers, no detailed pathological description are given.

Schern[4] in 1909 studied an epidemic among tame laboratory rats in which the obvious signs of illness lasted only two to three days. The disease was characterized by emaciation, loss of appetite, and in some animals by profuse diarrhea. The lesions found were injection of the peritoneum, without inflammatory exudate, marked enlargement of the spleen, cloudy swelling of the liver, with punctiform red areas beneath the capsule and on section. In the spleen and liver of animals which survived the infection for a longer period, there were pinhead-sized greyish or greyish-yellow foci, suggesting tubercles. The stomach was normal. The intestine was distended, the mucosa in the greater number of cases swollen and congested. The Peyer's plaques were conspicuous. Kidneys were cloudy and swollen, the adrenals slightly reddened. In two rats, large nodules suggesting "pseudo-tubercles" were present in the lungs.

Steffenhagen[5] in 1911 made a careful study of the gross lesions in rats fed with the Liverpool virus, which he considers identical with *B. enteritidis*. Like all the observers quoted, he records marked enlargement of the spleen, which is described as greyish black or reddish grey, with a firm "amyloid-like" consistence; rarely, there was found a soft, hyperplastic splenic tumor. The intestines were distended, the venules of the serosa and mesentery injected. The small intestine contained pale-yellow or thin dark-brown fluid, in which red blood cells could be found microscopically. The mucosa was unchanged but the follicles were swollen. The large intestine was not altered. The lungs were dirty grey on section, very bloody, and succulent. There were pinhead-sized hemorrhages beneath the pleura, and often greyish nodules suggesting tubercles. These consisted of necrotic areas with round-celled infiltration.

[1] *Op. cit.* [3] *Op. cit.*
[2] *Op. cit.* [4] *Op. cit.* [5] *Op. cit.*

Here and there were found pepper-grain sized abscesses with minute metastatic abscesses in the surrounding area. Bacilli were not found in smears from these, so that the origin of the pulmonary lesions was questionable. The liver was enlarged, brownish red, bloody on section, showing irregular, yellowish patches; in advanced cases, the entire liver was of a bright yellow color save for small reddish patches. The cervical lymph-nodes were found enlarged in one case. The adrenals were either diffusely hyperemic or showed a hyperemic zone about the medullary portion.

Boycott[1] in 1911 made the interesting observation that infection of white rats with the Gaertner bacillus was frequently accompanied by methemoglobinemia. The other lesions noted by him, were necroses of the liver and spleen, and, in five rats, acute suppurative myocarditis of the left ventricle.

The foregoing citations include, we believe, the more important contributions to the pathology of the disease caused in rats and mice by bacilli of the paratyphoid and enteritidis group. To this list should be added the study of the liver lesions in rabbits and white mice following infections with *B. suipestifer*, by Boxmeyer,[2] and the recent work of Ordway, Kellert, and Huested, also with a strain of the hog-cholera group. These we shall consider in discussing the genesis of the liver lesions.

2. Gross Lesions.

The gross lesions noted in the present epidemic agree quite closely with those recorded by the writers cited above. No differences were observed between the spontaneously and the artificially infected rats, nor between those infected by subcutaneous injections and those in which the disease was reproduced by feeding. The intestinal lesions, in particular, were not more striking in animals infected by the intestinal route.

Emaciation was always more or less marked. The peritoneal cavity was always free from exudate, even in rats injected intraperitoneally. Marked hyperemia, as described by Bahr and Schern, was not seen. The liver was pale and friable, and sometimes mottled. In some cases, minute, translucent, greyish foci could be detected with the magnifying glass, but were not visible to the unaided eye. The spleen was greatly enlarged in all cases but one, in which it measured only 27 mm. as against 33 mm. and 32 mm. in the healthy controls of the same litter. This exceptional finding will be discussed later. A typical example is Litter D, in which the infected spleens measured 37, 40, and 39 mm.; that of a healthy control rat of the same litter, killed at the same time, 23 mm. The spleens were firm and tense, very dark bluish purple, but irregularly mottled on section by hemorrhagic and greyish areas.

[1] *Op. cit.*

[2] *Jour. Med. Research*, 1923, N. S., 4, p. 14[illegible].

The stomach showed nothing abnormal. The intestines were usually collapsed. The intestinal contents in those animals which had previously had diarrhea were fluid, sometimes admixed with mucus, and occasionally flecked with bright blood. Other animals showed normal intestinal contents, with formed scybalae in the large gut. A few scattered petechial hemorrhages were occasionally seen, but no lesions which could be justly described as a hemorrhagic enteritis. The appearance of the Peyer's plaques varied. In the majority of the cases, they were pale and not abnormally prominent. In one case, only, was there found a small ulcer, and this proved on microscopical examination to be a localized destruction of mucous membrane by a rather extensive submucous hemorrhage.

The mesenteric lymph-nodes were not noticeably enlarged, and were not hyperemic. The lungs, with the exception of occasional small purpuric spots beneath the pleura, showed no gross change. One rat, which had been infected by feeding, showed marked alterations in one lung, the relation of which to the inciting organism was not proven. The lung in this case was shrunken, firm, and riddled with caseous abscesses of large size. Microscopically, there was an interstitial pneumonia, and bronchiectatic cavities filled with necrotic exudate. Whether this isolated finding is related to the disease, we are unable to say, as no bacteriological examination of the lung was made, altho the organism was readily recoverable from the blood and spleen in this case. The lesion is apparently the same as that described by Schilling,[1] and according to Currie,[2] is a not infrequent finding in rats apart from the disease which we have studied. The fact that a similar bronchiectatic cavity, lined with squamous epithelium, was found in a rat killed three days after infection would make it improbable that the lesion is associated with the disease.

The heart muscle was sometimes pale, and in one spontaneously infected rat showed macroscopically visible opaque, greyish areas in the left ventricle. The blood was often strikingly thin, and sometimes of a distinct brownish tinge.

Swelling of the cervical and mediastinal lymph-nodes was quite

[1] *Op. cit.*

[2] *Bull. No. 30, U.S. Pub. Health and Mar. Hosp. Serv.*, 1910, p. 55.

regularly found. No gross changes were observed in the kidneys, pancreas, testes, adrenals, or brain. The thymus was usually atrophied and the interlobular septa edematous.

3. Histological Lesions.

Liver.—In both spontaneously and experimentally diseased rats the liver was the seat of numerous focal necroses. These were present in all the cases examined, with the exception of three rats killed on the day following subcutaneous inoculation. The smallest and earliest lesions (30 hours after injection) appeared as agglomerations of distorted nuclei, lying in a fibrinous meshwork in which were entangled a few red blood cells. In the older lesions, the nuclear constituents gradually disappear; there remains a sharply outlined, roughly circular area, almost devoid of cells, staining intensely with eosin, and showing a swollen nodular framework of fibrin. In places the interlacing nodular strands of fibrin appear to fuse into hyaline masses. The fibrinous material stains orange red with Mallory's connective tissue stain, and bluish with Weigert's fibrin stain.

The necrotic areas are at once made evident, in sections stained with Sudan III, by the abundant accumulation of fat drops. These are of largest size and most numerous at the margin of the necroses. The central portion may contain a few finely divided droplets, or may be virtually free from fat. Here and there are small groups of liver cells laden with fat. These are probably at the edge of necrotic areas which did not appear in the sections. Fine fat droplets are also found free in the capillaries and within the Kupffer cells.

Apart from the necrotic areas, the liver cells show no degenerative changes. In some of the rats, notably in one killed 24 hours after subcutaneous inoculation, mitoses are very numerous, but not especially so about the necroses.

Within the liver capillaries, are found in great number abnormal cellular elements and occasional fibrin thrombi. The cellular structures are, for the most part, large mononuclear cells with basophilic cytoplasm, and relatively large, kidney-shaped nuclei. The fewest of these are normal and the greater number more or less

degenerated. They very commonly inclose pyknotic nuclear particles; sometimes the fairly preserved remnant of a lymphocyte, sometimes one or more red cells, rarely a group of bacilli. One finds also unphagocyted chromatin fragments and small cells with ring- or signet-shaped nuclei, obviously lymphocytes in various stages of

FIG. 1. -Liver of *Rat C1*, spontaneously infected, showing necrotic areas (*a*), and large masses of cells in a sub lobular vein (*b*)

pyknosis. In some of the sections, cellular elements and detritus of this sort are extremely abundant, and appear to block the capillaries, especially when they become inclosed in a fibrinous matrix. We shall discuss the relation of these cell fragment thrombi to the origin of the necroses farther on.

The behavior of the Kupffer cells was somewhat variable. In some of the rats they were swollen and vacuolated, sometimes exfoliated, and frequently contained inclusions of nuclear material or erythrocytes. In other rats showing equally extensive areas of necrosis, the Kupffer cells showed little or no change. In one infected rat which was vitally stained with Trypanblau very few

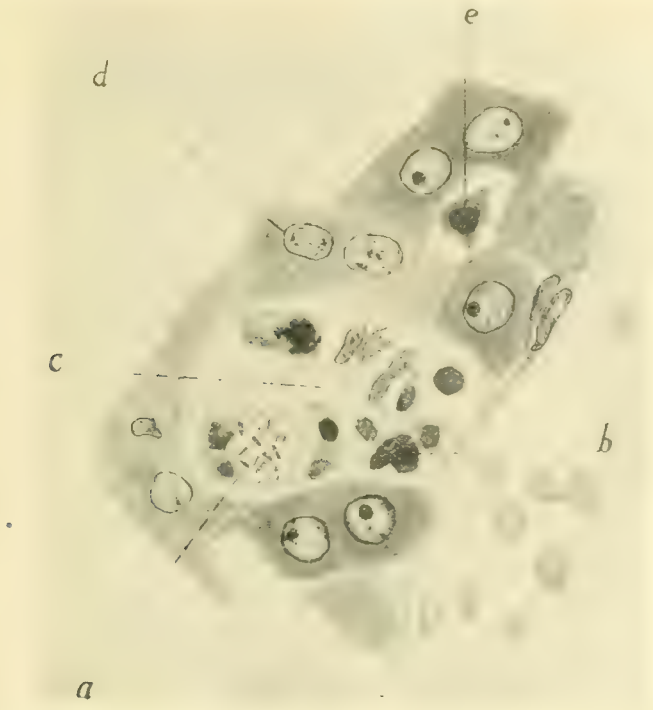

FIG. 2.—Early cell fragment thrombus in liver. At (*a*) mononuclear phagocyte enclosing bacilli; *b*) degenerating mononuclear cells; (*c*) fibrin net.

of the cells lying free in the capillary lumina contained blue granulae. There was no noticeable accumulation of the stained cells about the necroses or within them. Those endothelial cells which lay within the necrotic areas were obviously degenerated. The blue-staining material was in the form of large clumps instead of granulae of fairly uniform size. So far as one can judge from this single experiment, it would seem that the Kupffer cells are apparently not

the chief source of the foreign cellular elements and cell detritus in the capillaries.

All the different forms of cell fragments, phagocytes, and intact mononuclear cells are found in the portal vein, and at times in the efferent veins of the lobules. They occur singly or in large

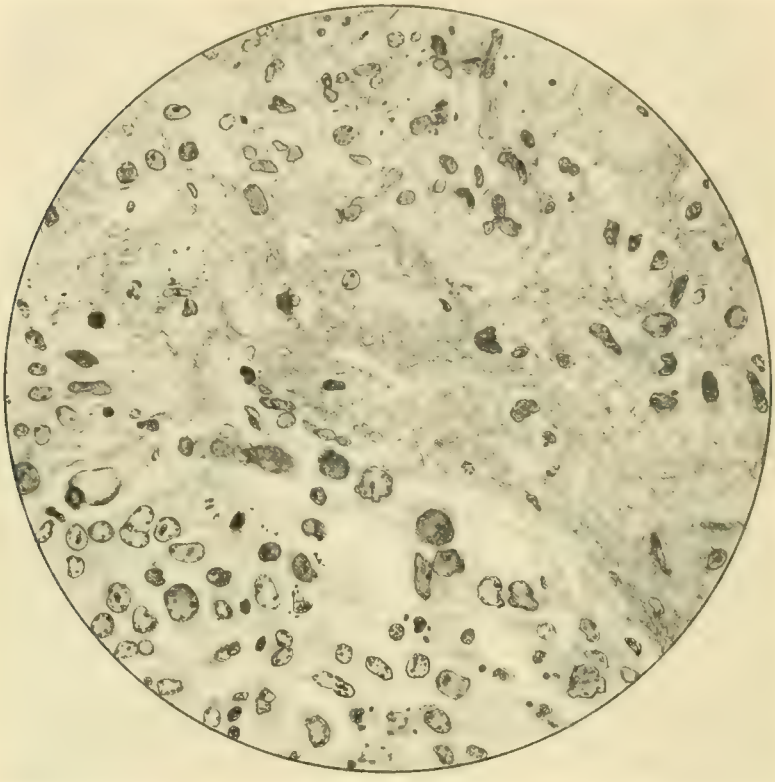

FIG. 3.—Spleen of *Rat B-2-x*, experimentally infected. Section includes edge of follicle. Note fragmentation of nuclei, especially in pulp, and abundant exudation of fibrin.

clumps, in which the cellular constituents are imbedded in a fibrinous matrix.

Spleen.—In this organ, the lesions are localized in the early stages, more diffuse in animals which survive the infection for a longer period. The essential, and probably the primary change appears to be an intense destruction of the lymphoid cells both with-

in and without the follicles. Throughout the pulp, one finds small mononuclear cells in all stages of disintegration, as evidenced by the abundance of fragmented chromatin. Much of this nuclear detritus is within macrophages, which in turn undergo degeneration. The necrosis of the pulp cells is accompanied by a great outpouring of fibrin, forming a nodular network about the degenerating cells. The filaments of fibrin appear to use the fibrous reticulum as a support. There is often distinct hemorrhage, but rarely excessive pigment. The follicles show invariably a more or less marked pyknosis of the small lymphoid cells. This is accompanied in the earlier stages by an excessive formation of large mononuclear cells. The number of mitoses among these may be extraordinary. Phagocytosis of chromatin fragments by the larger cells of the follicles is of course regularly seen. No fibrin could be demonstrated within the follicles.

Bacilli are found in small groups or in larger clumps both within and without the sinuses. In spontaneously infected rats, or in those infected by feeding, very few bacteria may be present. The sinuses are crowded with red cells, but they may contain impacted masses of mononuclear cells or cell-débris, about which there may be formed a definite fibrin thrombus. Masses of cells are also commonly found in the splenic veins. Among these are cells which may undergo mitotic division in the blood stream. Such cells were also found several times in smears made directly from the splenic blood, in which one finds also numbers of nuclear fragments and phagocytes enclosing red cells and chromatin particles.

Kidneys. In animals experimentally infected with subcutaneous or intraperitoneal injections, it is usual to find hyaline thrombi in the glomerular capillaries. Every tuft may show a more or less complete blocking. The appearance is especially striking in sections stained with Mallory's anilin blue, the hyaline material taking a reddish-orange color in contrast to the yellow of the red blood cells. Similar thrombi may be found in the capillaries of the medulla and pyramids.

Bacterial emboli in the glomerular loops and in the capillaries between the tubules were seen in two rats inoculated intraperitoneally, but were not regularly present.

Myocardium.—Lesions in the myocardium were found both in the spontaneously and experimentally infected rats, tho not in all cases. The changes were in the nature of an acute interstitial inflammation, and occurred by preference in the superficial strata

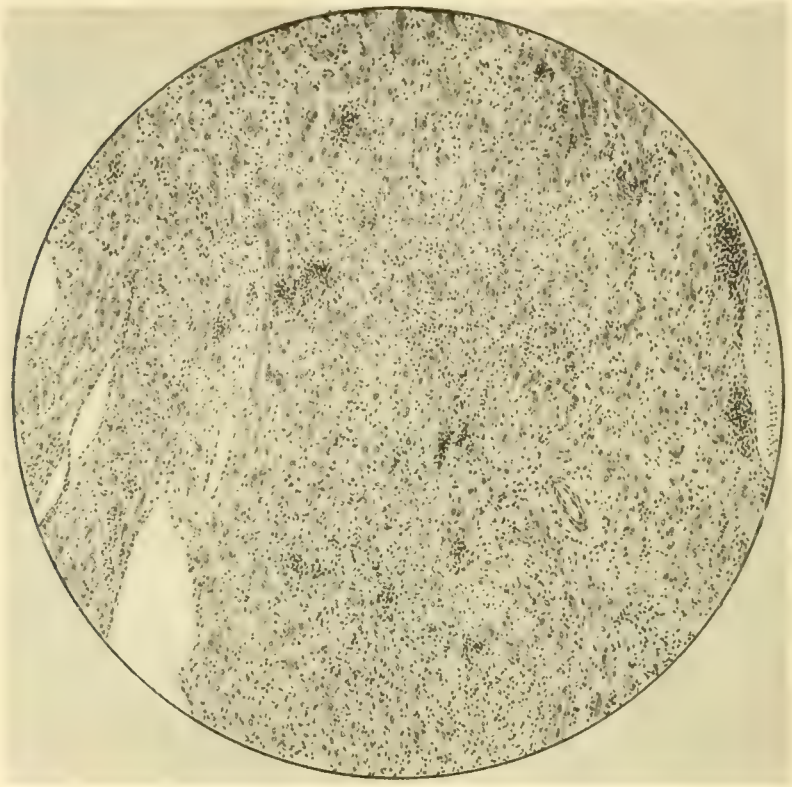

FIG. 4.—Myocardium of left ventricle, showing acute interstitial inflammation in spontaneously infected rat.

of fibers, more especially in the left ventricle. Rat A (spontaneously infected) showed extremely marked lesions (Fig. 4). The fibers were forced apart by a cellular exudate and fibrin. Most of the inflammatory cells are fragmented beyond recognition, but at the margin of the lesion, it can be seen that there is swelling and degeneration of the fixed connective tissue cells, as well as a moderate infiltration of lymphoid and polymorphonuclear elements.

The muscle fibers show various degrees of degeneration up to complete necrosis. In some areas, the fibers have disappeared completely, leaving only an irregular eosin-staining material, in which are found swollen, distorted nuclei and chromatin fragments. Bacilli occurring singly and in clumps are found without difficulty between the muscle fibers.

Lungs.—The septa appear relatively stout and more cellular than those of normal lungs. This is due to the stuffing of the capillaries with large mononuclear cells similar to those found in the liver. Many of the larger pulmonary arteries contain groups or larger masses of these cells. Pneumonic lesions, aside from these interstitial changes, were not found, and the bronchi were free from exudate. The one exception (Rat D2*x*) has already been described.

Stomach and intestines.—The only lesions found in the stomach were occasional small hemorrhages into the mucosa. The intestinal lesions, on the whole, were not striking, and in some of the rats no definite alterations were found. In others there was moderate fragmentation of the lymphoid cells, with phagocytosis by the swollen reticular elements. The overlying mucosa was usually intact, but not infrequently the villi were edematous, and there were more or less extensive hemorrhages; this had led in one case to a separation and desquamation of the epithelial covering; aside from this purely mechanical loss of substance, no ulcers were found in any of the rats examined.

Lymph-nodes.—Serial sections of the neck organs were made from a number of the spontaneously infected rats for another purpose. In all the cases it was found that the cervical lymph-nodes were greatly enlarged and the seat of extensive necroses similar to those described in the spleen. Altho the thymus exhibited advanced involutional changes such as are found in all infectious diseases accompanied by wasting, in no case did it contain areas of necrosis, even when all the surrounding lymphoid tissue, including that which abundantly envelops the primary bronchi, was extensively affected. The mesenteric lymph-nodes showed slight involvement in some rats; in others, no changes were present.

The marrow of the long bones contained more or less extensive

areas of necrosis (Fig. 5) distributed throughout the shaft. Necroses were also found in the bones of the jaw and clavicle. There seemed in some cases to be an increased number of megakaryocytes present, and in smears from the marrow

FIG. 5.—Section through shaft of femur showing extensive necroses in marrow of *Rat D-4-x*, spontaneously infected.

stained with Giemsa one of these cells containing plural mitoses was seen.

No lesions were found in the thyroid, parathyroid, pancreas, or testes. The adrenals showed no loss of chromaffinity and were normal in other respects. The central nervous system, which showed no gross changes, was not studied microscopically.

PROTOCOLS.

Rat C 1. This was one of a litter which had been operated upon when 14 days old. The animal gained normally until it became ill, when the weight dropped from 98 gm. to 77.5 gm. The rat became emaciated and refused food, and the eyes were closed with secretion. It was killed by chloroform.

Autopsy: No intestinal lesions were noted. The spleen was enlarged and showed prominent follicles. The liver was large and pale. No other gross changes were observed.

HISTOLOGICAL EXAMINATION.

Liver: Very numerous areas of necrosis, for the most part discrete, but in places confluent. The liver cells at the margin of these finely vacuolated. The necrotic areas are almost devoid of cells, being composed of a coarse fibrinous reticulum, in which are a few lobulated nuclei. In some places, small accumulations of leukocytes, chiefly polymorphonuclear. Dense aggregations of cells in the portal spaces, most of them lymphoid and plasma cells. These often extend in streaks along the interlobular bile-ducts and vessels. The liver capillaries are narrowed apparently by the swelling of the liver cells. They contain degenerated cells which in a few places form definite plugs.

Spleen: With the low power, one sees numerous scattered, rather ill-defined areas, less cellular than the surrounding pulp, composed of a hyaline or granular eosin-staining material deposited in the reticulum about the splenic sinuses. The cells which are included in these regions are pyknotic and fragmented. In places, distinct hyaline thrombi occupy the sinuses in the center of these rarefied areas. About such thrombosed sinuses there are often irregular hemorrhages. The malphigian follicles show slight changes, but phagocytosis of fragmented nuclei is occasionally seen. Mitoses are not numerous. A few megakaryocytes are scattered through the pulp.

Adrenals: Normal. The chromaffin staining is marked.

Kidney: Normal.

Lungs: The alveolar walls thickened showing nodular areas of necrosis. Many of the smaller arteries packed with masses of mononuclear cells.

Rat C-2.—Thymectomy at 14 days. Recovered from operation, and gained steadily in weight until 24 days before death. The weight then remained stationary for 10 days, but fell from 59 gm. to 43.5 gm. during the last two weeks of life. The animal was found dead and partially eaten by the other rats of the litter.

Autopsy: No gross lesions were noted, aside from the extreme emaciation. The spleen measured only 25 mm. in length.

HISTOLOGICAL EXAMINATION.

Liver: Necroses, very abundant and some of them of large size, are composed of a swollen hyaline fibrinous reticulum inclosing a sparse number of fragmented or distorted nuclei and a few red blood cells. The liver capillaries, which are narrow, contain many small chromatin fragments, but no very definite thrombi, nor are larger masses of cells found in the portal veins. The intact liver cells often show large, hyperchromatic nuclei.

Spleen: Very markedly altered, the follicles small and very irregular. The small cells of the follicles are in all stages of pyknotic degeneration. Many large phagocytes filled with nuclear fragments. The pulp shows a striking paucity of cells as compared

with the normal. Between the compressed, and in places, obliterated sinuses, there is a large amount of a granular fibrinous material, imbedded in which are nuclear fragments and red blood cells. The endothelium of the sinuses is well preserved, but the cellular constituents of the pulp are largely disintegrated. Some of the larger sinuses contain hyaline thrombi, others are filled with loose masses of degenerating cells.

Adrenals: Almost complete loss of chromaffin staining, possibly the effect of postmortem change. The cortex is not altered.

Myocardium: In the wall of the left ventricle several small localized areas of hyaline or waxy degeneration of the muscle fibers, and about these, slight cellular infiltration.

Lungs: No noteworthy changes.

Cervical lymph-nodes: Numerous areas of necrosis, located chiefly in the lymph-cords.

In this animal, the disease was apparently of long standing, and had led to extreme destruction of the cellular elements of the spleen, and to very extensive necroses in the liver. There was, however, no attempt at healing or encapsulation, and the lesions may be considered as progressive. An exceptional feature in this rat is the small size of the spleen, due possibly to the advanced destruction of the cellular constituents, or to a secondary contraction of the necrotic areas, such as occurs in an anemic infarct.

The third rat of this litter illustrates the more acute lesions.

Rat C-7.—A control rat, unoperated, which gained weight progressively until killed, showing no abnormal symptoms.

Autopsy: Weight 70.5 gm. The organs appeared normal with the exception of the spleen, which measured 35 mm. in length. The follicles were very conspicuous.

HISTOLOGICAL EXAMINATION.

Liver: A few typical early necroses, some of which are the seat of marked leukocytic infiltration. Definite hyaline plugs are found in some of the necrotic foci. The small sub-lobular veins are surrounded by a mantle of lymphoid and plasma cells. Swelling and some vacuolization of the liver cells. The capillaries are narrow and contain moderate numbers of small cells, but no larger masses of cell fragments, or phagocytes.

Spleen: The pulp intensely congested, and in places, hemorrhagic. A few small areas of necrosis, with fragmentation of the nuclei. The follicles are large, the follicle cells well-preserved. Numerous megakaryocytes are present.

Adrenals: Intense chromaffin staining.

Thymus: Normal.

Rat E-6.—Unoperated control, aged 2 months and 6 days. Duration of disease as estimated from beginning loss of weight was 9 days, during which period the weight fell from 65.5 gm. to 59 gm. The rat was chloroformed, having shown the usual symptoms.

Autopsy: Emaciated. No peritoneal changes. Spleen much enlarged (36 mm.), with conspicuous follicles. Liver shows nothing abnormal. Adrenals, kidneys, lungs, intestines free from gross changes.

HISTOLOGICAL EXAMINATION.

Liver: Sharply circumscribed, very numerous necroses, some of large size, composed of a coarse fibrinous meshwork with occasional red blood cells, and degenerating

cells and nuclear fragments. Such cells are also found free in the capillaries, singly or massed into clumps and enveloped in a delicate fibrinous mesh. The Kupffer cells in places are swollen and contain ingested red blood cells and chromatin particles. Aggregations of large cells in the portal veins and often in the lymph-spaces of Glissons capsule.

Spleen: The section shows numerous scattered areas of necrosis, consisting of a fibrin meshwork inclosing distorted, pale, epithelioid nuclei, erythrocytes, and small particles of fragmented nuclear material. Elsewhere the sinuses are congested and frequently contain masses of large mononuclear cells, few polymorphonuclear leukocytes, and other cell fragments whose origin it is impossible to determine. Definite thrombus formation is not noted. Mitoses numerous, both within the follicles and in the reticulum. A great many megakaryocytes.

Lungs: Throughout the section, irregular, somewhat nodular areas of consolidation, not of the type of a lobular pneumonia, but consisting of cellular infiltrations or proliferations of the alveolar wall, without exudation into the alveoli. In the center of such an area, there may often be found a small pulmonary artery packed with cells which are of the same kind as those seen in the liver capillaries. Large mononuclear cells predominate. They are often vacuolated, and frequently inclose erythrocytes or pyknotic nuclear fragments. The consolidated areas are thus rather definitely distributed about the smaller arterial branches, and not the bronchi which are free from exudate and otherwise quite normal. The capillaries in these areas are not infrequently found plugged by fibrin masses inclosing nuclear fragments.

Myocardium: Most extensive changes (Fig. 4). The fibers are widely separated, there having been evidently an extreme edema of the interstitial tissue. The muscle fibers are thin, and very refractile and waxy in appearance, with complete loss of the transverse striations, and only a suggestion here and there of the longitudinal fibrils. The nuclei are for the most part still fairly well preserved, a few only being pyknotic. Between the fibers are accumulations of wandering cells, the majority of the mononuclear variety. In places, and especially beneath the endocardium, they form dense clusters. There appears to be also a proliferation of the fixed connective tissue cells; altho no mitoses are found, many of the cells between the degenerated fibers are of the fibro-blastic type. These myocardial changes are not uniformly present throughout the heart, but affect particularly the wall of the left ventricle and the papillary muscles.

Organs of the neck: These were cut in series of 10 microns. The thymus was the seat of marked involutional changes. Differentiation between cortex and medulla was lost. The lobules were separated by edematous tissue in which were many mast-cells. The mediastinal and cervical lymph-nodes were enlarged and contained many areas of focal necrosis. The thymus, on the other hand, was entirely free from necroses. The thyroid and parathyroids were normal. The salivary gland was normal.

Bone-marrow (femur): Scattered areas of focal necrosis, distributed throughout the shaft and present also in the lower epiphysis. Megakaryocytes appear to be present in increased numbers.

In all, 10 spontaneously infected rats were examined and a fairly complete microscopic study made. From the last rat examined, and from several other rats infected at about the same time, the bacillus which has been described above was isolated, and a further

study made of the lesions produced by experimental infection with pure cultures of the organism. As has been stated, it has been found to be easy to produce the disease, either by feeding or by subcutaneous or intraperitoneal inoculation. The resulting lesions were in all respects similar to those described in the spontaneously infected rats; the differences found in rats infected by different routes appeared to depend upon the duration of the illness rather than the mode of infection.

The following protocols of experimentally infected rats illustrate the similarity between the naturally and artificially produced disease:

Rat B-1-x.—Inoculated intraperitoneally with 0.5 c.c. of a saline suspension of a 24-hour agar culture. Found dead 48 hours later. No putrefactive changes.

Autopsy: Negative save for slight swelling of spleen. Cultures from peritoneal cavity, heart blood, spleen, and liver, positive.

HISTOLOGICAL EXAMINATION.

Liver: Many small areas of necrosis, scattered through the different portions of the lobules. The smallest, and presumably the earliest of these, appear as irregular plugs of coarse fibrin distending the capillary lumen and inclosing numerous unaltered red blood cells and a few nuclear fragments. In the capillaries are many mononuclear cells, with inclusions of masses of chromatin and red cells (Fig. 2). The Kupffer cells, swollen and vacuolated, also contain ingested nuclear particles and erythrocytes. The origin of most of these phagocytic cells, which are in all stages of disintegration, is impossible to determine. It is obvious that about these masses of cell detritus fibrin thrombi are formed in the sinusoids, and that these cell-fragment thrombi antedate the destruction of the liver cells. Thus one finds occlusion of the capillaries without noticeable alteration of the adjacent liver cells. With the stoppage of a larger capillary area, the liver cells about the thrombosed area become vacuolated, their cell outline indistinct, their nuclei shrunken and irregular in outline; the nucleolus disappears, and the chromatin lies against the nuclear membrane or is extruded into the cytoplasm. With the complete breaking-down of the liver cells, they become incorporated into the thrombotic area.

Many large mononuclear cells, most of them showing karyolytic changes, are present both in the portal and in the efferent veins. Many of the individual cells are phagocytic.

Bacilli are found in clumps in the capillaries, but in no particular relation to the necroses.

Spleen: The follicles irregular, but large and relatively well preserved. The most striking alteration is in the pulp, which even with the low power shows an extraordinary fragmentation of the nuclei (Fig. 3). The meshes of the reticulum are thickly crowded with nuclear particles of all sizes and shapes between which there is hemorrhage and fibrin formation. The picture is complicated, but it is evident that there has been an extreme destruction of the lymphoid cells, both within the follicles and in the reticulum. Coincidently there is taking place a proliferation of large mononuclear cells, with lobate or oval nuclei and basophilic cytoplasm. The follicles con-

tain many healthy appearing cells of this type, and many of them are in mitosis. They become phagocytic for the pyknotic, small lymphoid cells and after becoming loaded with ingested nuclear material, in turn degenerate. The larger sinuses and veins contain many of these cells and some of them appear to undergo mitosis in the circulating blood. Some of the sinuses are plugged with cell-fragment thrombi, but the majority are filled with well-preserved red blood cells. The endothelium of the sinuses preserves its normal character, shows no mitoses, and contains no phagocytic inclusions. No pictures are found suggesting a differentiation of the sinus endothelium into the large basophilic mononuclear type of cell.

Large colonies of bacilli are scattered irregularly through the splenic pulp. No megakaryocytes are seen in the sections.

Kidneys: Hyaline thrombi present in the glomerular capillaries, forming more or less complete casts of the tufts. They stain intensely with Weigert's fibrin stain. The blocking of the blood-channel is not always complete, the hyaline or fibrinous material being sometimes deposited along one side of the capillary wall. The epithelium of the tuft and of Bowman's capsule unaltered. Occasional hyaline thrombi also found in the capillaries between the collecting tubules. The thrombus may extend for a distance either into efferent or afferent vessel or both.

Adrenals: Normal in all respects.

Intestines: A few hemorrhages into the mucosa. The sections do not pass through the Peyer's plaques. The serosa is free from inflammatory change.

Stomach and duodenum: Normal.

Pancreas: Normal.

Mesentéric lymph-nodes: There is a slight hemorrhage near the surface of the gland, but no necrosis. The changes are insignificant.

Lungs: By oversight, not examined. Section of lung from Rat B-2-*x* inoculated at same time and showing similar lesions in other viscera, may be taken for description. The capillaries are crowded with degenerating cells of the same character as those seen in the liver capillaries. Large mononuclear cells often enclosed in a delicate fibrin network, are also present in numbers in the smaller pulmonary arteries, but are rarely found in the veins. Bronchi and alveoli are normal.

Myocardium: The superficial layer of ventricular muscle is edematous. The fibers are separated by a fibrinous coagulum. There are bacterial emboli in the capillaries.

The following protocol describes the lesions in a rat infected by feeding.

Rat D-1-x.—Fed with cubes of bread soaked in saline suspension of 24-hour agar slant. No food given on following day. The rat showed the usual symptoms and died nine days after infection. On ice, six hours before autopsy.

Autopsy: Marked emaciation. Hyperemia of intestine without marked swelling of Peyer's plaques. Spleen much enlarged, liver moderately. Lungs and heart show no gross changes.

HISTOLOGICAL EXAMINATION.

Liver: Necrotic areas of considerable size present in great numbers, irregularly disposed in various portions of the liver lobules. In these areas, the architecture of the liver is destroyed; one sees merely a very coarse nodular network of fibrin in which lie a few distorted nuclei and nuclear fragments. Some of these appear to have been derived from the pre existing endothelial cells; others are small and round and resemble

pyknotic lymphocytes. The liver cells immediately abutting on the necrotic areas sometimes show nuclear degeneration, but on the whole, the transition to normal tissue is very abrupt. Apart from the necrotic areas, one finds as in Rat B1*x*, distended capillaries filled with broken-down cellular detritus, enmeshed in fibrin, and about these, changes in the liver cells of varying degree up to complete necrosis. Very striking are the large masses of cellular detritus in the larger efferent veins.

In sections deeply stained with polychrome methylene blue, very few bacilli are found, and these scattered irregularly through the capillaries. They are often inclosed within phagocytes and degenerated. Larger bacterial colonies are not seen.

Spleen: The lesions are less marked than in many other rats examined. The follicles are small and indistinctly outlined. Fragmentation of the lymphoid cells and phagocytosis are not extreme. The changes are more pronounced in the reticulum. The sinuses which are distinct and well filled with red blood cells are widely separated by a fibrinous material, containing distorted nuclei, in different stages of disintegration. Some of the larger sinuses, but especially the larger splenic veins, are filled with masses of cells embedded in a pink-staining matrix.

Kidneys and adrenals: No marked change. No fibrin thrombi in the glomeruli.

Lungs: Lesions are practically identical with those found in Rat B2*x*.

Stomach: Localized areas of intense hyperemia in the superficial layers of mucosa, and red cells are found in numbers in the stomach contents.

Intestines: No changes are found in the mucosa of the small or large intestine. The Peyer's patches are not included in the sections.

Mesenteric lymph-nodes: Quite numerous areas of partial necrosis, in which the lymphoid cells are replaced by large cells with vesicular lobate nuclei, often containing pyknotic nuclear particles in their cytoplasm.

Myocardium: Normal.

In all, 22 rats were inoculated, and a histological study made of the lesions. The above protocols are selected as typical of the early and late stages of experimental infection. They do not, however, include a description of the changes in the Peyer's plaques, a brief record of which may be taken from the protocols of other infected rats.

Rat M-5-1.—Subcutaneous injection. Killed after 47 hours. Very early lesions in spleen and liver.

Stomach: Normal.

Small intestine: The epithelium is intact. Among the lymphoid cells of the Peyer's plaques are very numerous cells of large size, with vesicular, slightly irregular or lobulated nucleus. These cells are in active proliferation: mitotic figures extremely abundant. The small cells well preserved. Only a few pyknotic nuclei.

The only abnormality noted in these sections is the very active proliferation of large mononuclear cells, unaccompanied by marked destruction of the lymphocytes, or phagocytosis.

Rat M-5-2.—Subcutaneous injection. Killed 57 hours after inoculation. Typical marked lesions in liver and spleen. The changes in the small intestine are similar to the above, but mitoses among the large cells are less numerous.

Rat M-12-4.—Splenectomy. Subcutaneous infection. Killed 3 days after inoculation. Typical lesions in the liver.

Small intestine: The changes very slight, and of the same character as in Rats M-5-1 and M-5-2. No necroses or hemorrhages. The mesenteric lymph-nodes show most active proliferation of large mononuclear cells. The sinus endothelium is readily distinguished from these, and tho there is slight swelling and desquamation, no active proliferation is seen.

Rat M-12-5.—Subcutaneous inoculation. Killed after 3 days. Typical lesions in spleen and liver.

Intestines: Peyer's patches large. In a few places, pyknosis of lymphoid cells, with phagocytosis. The large cells show numerous mitoses. The overlying epithelium is unchanged. In some sections, however, there is hemorrhage into the mucosa, with edema as shown by the separation of cells of the stroma and the deposition of granular material. No inflammatory reaction. Sections of several taenia in the villi.

The above brief notes will suffice to show the insignificance of the intestinal lesions. Aside from moderate fragmentation of lymphoid cells, active proliferation of mononuclear elements apparently derived from the reticulum, no changes of note were observed. The presence of parasites in the intestinal villi in Rat M-12-5 may perhaps explain the more marked hemorrhage and edema seen in this case.

LESIONS IN MICE.

The following is a brief abstract of the changes noted.

Mouse 1.—Inoculated intraperitoneally with 0.3 c.c. of a saline suspension of a 24-hour agar slant. Found dead after 18 hours.

Autopsy: Spleen enlarged. Liver pale. Lungs congested. Many bacilli in heart blood and peritoneal exudate.

HISTOLOGICAL EXAMINATION.

Liver: Fibrinous exudate with large masses of bacilli on the surface. Capillaries contain increased numbers of lymphocytes with occasional pyknotic nuclei. No thrombus formation. A few colonies of bacilli in the capillaries.

Spleen: Marked degeneration of the lymphoid cells, both in follicles and in reticulum, with extremely active phagocytosis. There are also extensive hemorrhages into the splenic pulp. Many pigment-containing cells. Many megakaryocytes. No definite exudation of fibrin.

Kidney: Many bacterial emboli in the glomeruli and elsewhere. The glomerular tufts are free from fibrinous thrombi.

Myocardium: Bacterial emboli are present in the capillaries.

Mouse 2.—Inoculated with 0.3 c.c. of saline suspension subcutaneously. Found dying after 18 hours. Killed with chloroform.

Autopsy: Spleen enlarged and congested. No other gross lesions. Many bacilli in smears from heart blood.

HISTOLOGICAL EXAMINATION.

Liver: Same as Mouse 1. No necroses or capillary thrombi.

Spleen: Same as Mouse 1, save that large mononuclear cells in mitosis are very numerous, probably because of fresh preservation. Many megakaryocytes.

Kidneys, lungs, and myocardium are normal.

In both mice, death occurred apparently without the development of marked lesions comparable to those seen in infected rats. A third mouse, inoculated with killed cultures, exhibited marked lesions, which closely resembled those in infected rats.

Mouse 3.—Inoculated intraperitoneally with 0.3 c.c. of culture killed by heating. Found dead after 18 hours.

Autopsy: Spleen markedly enlarged. Liver soft and pale. Other viscera show no changes.

HISTOLOGICAL EXAMINATION.

Liver: The capillaries are packed with chromatin fragments and distorted nuclei of bizarre shape. In many places, these are associated with the formation of definite fibrin thrombi. Necroses of the liver cells are seen in many places, but the appearance differs somewhat from that found in rats in the preservation of the alignment of the necrotic cells. Their nuclei at first become pyknotic, later fragment and disappear; the cells become smaller, denser, more hyaline, and stain intensely with eosin. The necrotic areas are not very circumscribed.

Spleen: Many fairly limited areas of necrosis in the pulp, in which there is extreme nuclear fragmentation. Hemorrhages also.

Lungs: Marked hyperemia. Capillaries stuffed with nuclear fragments.

Mediastinal lymph-node: Fragmentation of lymphocytes and phagocytosis of cell detritus by large cells. Exudation of coagulable material into the lymph sinuses.

Myocardium: Normal.

No feeding experiments were performed with mice, and the intestines were not examined.

LESIONS IN RABBITS.

As was stated in the bacteriological part of this paper, rabbits were found to be susceptible to intraperitoneal and intravenous inoculations with the bacilli. The lesions produced resemble closely the lesions observed in rats, as will be evident from the following protocol.

Rabbit 1.—Inoculated intraperitoneally with 1 c.c. of saline suspension of 24-hour agar slant. Died in 18 hours.

Autopsy: Along lymphatics of anterior abdominal wall, several small cheesy abscesses. No free fluid in peritoneal cavity. The omentum is rolled upon itself and dotted with opaque, whitish exudate. The serosa is not greatly congested. There is fibrino-purulent exudate of a cheesy character on liver, spleen, and intestinal serosa. Liver very large, dark, with distinct lobules having a darker center. The consistence is friable. No macroscopic necroses. No coccidiosis. Spleen moderately enlarged,

dark, firm. Kidneys very soft, friable, and swollen, sprinkled superficially and on section with blotchy hemorrhages. Adrenals: medulla hyperemic. Pancreas normal. Lungs: small pleural and parenchymal hemorrhages. Myocardium, stomach, and intestines normal. Mesenteric lymph-nodes not enlarged or necrotic.

Positive cultures obtained from peritoneal cavity and heart blood.

HISTOLOGICAL EXAMINATION.

Liver: The liver cords are narrow, the capillaries wide, and filled with great numbers of cellular fragments. In many places they are agglomerated into impacted masses imbedded in fibrin, which is readily demonstrated with Weigert's stain. The appearance is identical with that repeatedly described as typical of the livers of infected rats. In addition, however, there are larger areas which have rather the appearance of irregular infarcts. The liver cells still maintain their columnar alignment, but the individual cells are homogeneous and stain intensely with eosin. The nuclei are lost, and the cells evidently completely necrotic. The blood channels in these areas are not occluded with thrombi, but in some places are plugged with masses of bacilli. The entire necrotic portion which involves several lobules, is walled off by a zone of leukocytic infiltration. In the absence of serial sections, it was not determined whether there was a blocking of larger vascular channels.

Spleen: The striking feature is the presence of dense thrombi composed of fibrin, red blood corpuscles, and nuclear fragments in many of the splenic sinuses. There are areas of hemorrhage in the pulp. The follicles show changes which are the counterpart of those noted in rats.

Lungs: There is an unorganized fibrin thrombus in one of the larger branches of the pulmonary artery. This does not show the structure of a typical platelet thrombus, but appears to be composed of irregular laminae of fibrin, and contains dense aggregations of chromatin dust, in its central portion. Similar thrombi are present in smaller arterial branches near the surface and are associated with hemorrhage into the alveoli. The capillaries are crowded with leukocytes, among them many polynuclears. There is no exudate into alveoli or bronchi.

Stomach: Normal.

Small intestine: Sections include a Peyer's plaque. The mucosa is unchanged. Rarefaction of the centers of the follicles and fragmentation of the nuclei of the lymphocytes leading to their complete disappearance in some regions. Their place is taken by large mononuclear cells, also in various stages of degeneration, and containing yellowish pigment.

Myocardium, adrenals, and kidneys show no changes.

V. GENERAL CONCLUSIONS.

The disease, as exemplified by the foregoing description, presents features which are of considerable interest to the pathologist. The origin of the necroses in spleen, liver, lymph-nodes, and bone-marrow, which constitute the most striking alterations, demands explanation. Mallory and Ordway have already emphasized the resemblance of this rat disease to human typhoid in the character

of the lesions, and its suitability for the experimental study of some of the disputed points in the pathology of typhoid fever.

Two divergent opinions as to the origin of the liver necroses in human typhoid have been expressed. It has been held, on the one hand, that the lesions are the direct result of the action of bacterial toxins or of the bacteria themselves upon the parenchymal cells. Thus Reed[1] concludes, from a study of human lesions and necroses produced by the injection of typhoid bacilli into the mesenteric veins of rabbits, that the areas of necrosis "owe their origin to the action of the typhoid bacillus, altho it has not been possible to determine definitely in what way this cell death is brought about, that is to say, whether it is due to the immediate presence of the bacilli within the areas of necrosis, or is caused by the action of the so-called toxalbumins which are assumed to be present in the general circulation." He considers the latter more probable because of the experiments of Welch and Flexner[2] with diphtheria toxin and of Flexner[3] with minute injections of ricin.

The alternative view is that of Mallory,[4] who holds that the liver necroses in typhoid fever are the direct result of the embolic occlusion of liver capillaries by impacted masses of cells and cell-fragments, largely of endothelial origin ("endothelial leukocytes"), which are swept into the portal circulation from the spleen, intestine, and mesenteric lymph-nodes. The typhoid toxin acts primarily as a direct stimulant to the proliferation of the large mononuclear cells in these tissues. These new-formed cells are short-lived, and in their disintegration give rise to the cell fragment emboli in the liver and other organs.

In the lesions described, we have repeatedly noted the presence of great numbers of cell fragments in the liver capillaries, and of larger masses of cells in the portal vessels. Altho the appearances seemed to point to a capillary occlusion as the primary event, and the necrosis of the liver cells as a later effect, it seemed of interest to establish this point experimentally, if possible.

The first question to be determined was whether, in the earliest lesions, there could be shown a complete blockage of the capillaries

[1] *Johns Hopkins Hosp. Rep.*, 1895, 5, p. 379.
[2] *Johns Hopkins Hosp. Bull.*, 1892, 3, p. 17.
[3] *Jour. Exper. Med.*, 1897, 2, p. 197.
[4] *Ibid.*, 1898, 3, p. 611.

in the necrotic areas. Rats were infected by subcutaneous inoculation, and after varying intervals, india-ink was injected into the portal vein. In this way there was readily produced a diffuse and uniform injection of all the liver capillaries. The necrotic areas, however, remained completely uninjected, as shown in Fig. 6.

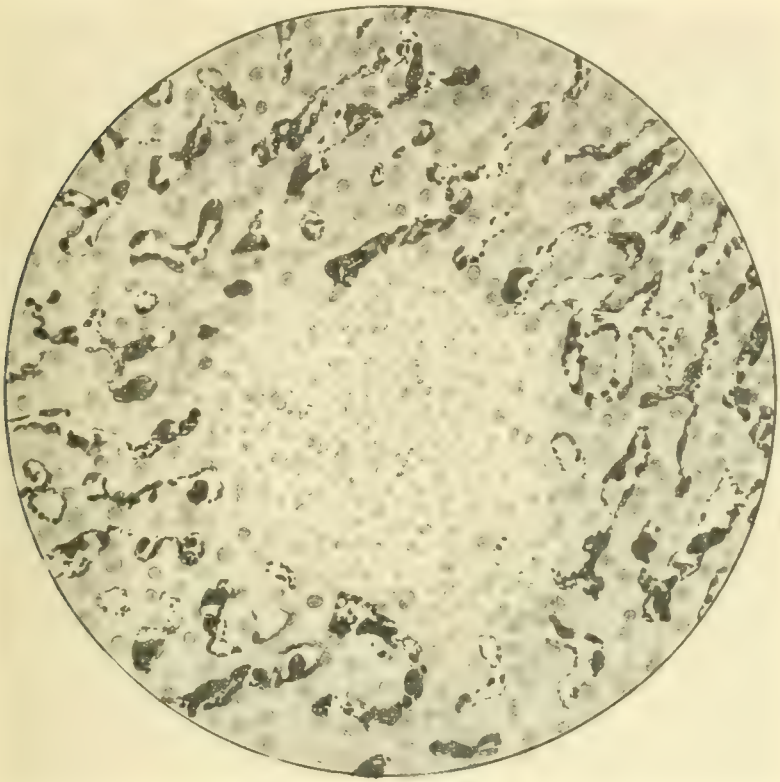

FIG. 6.—Liver of experimentally infected rat after injection of india-ink into portal vein. The necrotic area is impervious to the injection fluid.

and stood out conspicuously from the surrounding injected liver tissue. The capillaries in even the smallest and presumably the earliest necroses could be shown in serial sections to be absolutely impermeable to the injection fluid.

This fact, it seems to us, argues against a primary toxic injury to the epithelial cells, followed by a secondary thrombotic occlusion;

for, in such an event, one would expect to find lesions in which the capillaries were at least partially permeable to the injection fluid.

Because of the profound destruction of cells in the spleen, it seemed probable that the cell fragments found in the liver capillaries might be largely derived from this source. The fact that the intestinal lesions were relatively slight also seemed to bear out this idea. The same view had been previously expressed by Boxmeyer, who, in discussing the source of the large mononuclears found in the liver capillaries, stated that "in the mouse . . . . it is safe to conclude that by far the greater number arise in the spleen and are carried to the capillaries of the liver."

To test this point, we removed the spleens from two rats, infected them, together with controls, and killed them after three days. The liver necroses were of the same character in both sets of animals, and were associated with the formation of fibrin thrombi about cell-fragments. If anything, the necrotic areas were more numerous in the splenectomized rats than in the controls. From these experiments, it would seem that the presence of cells derived from the spleen is not essential for the development of the liver lesions.[1]

That necrotic cells, cell-fragments, and phagocytes are discharged from the spleen and are held back by the capillaries of the liver and spleen could be shown not only by finding such cells in masses in the afferent veins of the liver and in the pulmonary arteries, but by comparing smears made directly from the splenic vein with smears from the left ventricle.

The following count will serve as an example:

Rat M-5-2.—Injected subcutaneously. Killed after 57 hours. Typical gross and microscopic lesions.

Smear from splenic vein:	Per cent
Polymorphonuclear neutrophiles	26
(Of these, 10 showed degeneration, i.e., vacuolization of cytoplasm, loss of granules, indistinct nuclear outline, pale staining of nucleus; some of the leukocytes contained bacilli.)	
Small lymphocytes	11
(Of these, 2 showed distinct pyknosis, with irregularity of nucleus.)	
Large lymphocytes	14
(Of these, 3 showed indistinct nuclear outlines, pale staining, etc.)	

[1] Attempts to ligature the portal vein and thus to prevent the embolism of cells from the intestinal tract as well as the spleen, were unsuccessful, the rats surviving the operation only three to four hours.

	Per cent
Large mononuclear cells	18
(Characterized by round or reniform nucleus with distinct nucleolus, abundant faintly basophilic cytoplasm often studded with purplish granules. Many of these cells inclose one or several bacilli. One cell found undergoing mitosis.)	
Degenerated cells, unclassified	31
(Degenerated cells of various types, some mononuclear, some apparently derived from swollen polynuclears. They often enclose more or less disintegrated bipolar bacilli and occasionally nuclear fragments.)	
Smear from heart blood (left ventricle):	
Polymorphonuclear neutrophiles	34
(Of these, 5 showed degeneration. Several inclosed one or several bacilli, one a red blood cell, one a nuclear fragment of normoblast or lymphocyte.)	
Small lymphocytes	9
Large lymphocytes	31
Large mononuclears (not degenerated)	18
Degenerated cells, unclassified	8

In the smear from the splenic vein, no less than 46 per cent of all the leukocytes showed distinct evidence of degeneration, as against 13 per cent in the blood from the left ventricle. A similar difference was found in other infected rats. Altho the classification of many of the cells found in the smears may be open to question, the contrast is sufficiently striking to indicate that degenerated cells, and particularly phagocytes with bacillary or nuclear inclusions, pass out from the spleen and are held back by the capillaries of the liver and lungs from the general circulation —at least in part.

Since some of the phagocytes contain bacilli, it is difficult to say with certainty that the bacilli or their disintegration products are not concerned in the production of the liver lesions. Even if, as we believe, the lesion begins as a cell-fragment thrombus in the capillaries, it is readily conceivable that the death of the adjacent liver cells is due, not so much to a mechanical obstruction of the circulation, as to the toxic influence of bacilli included in the thrombosed area. Too much emphasis should not be laid upon the failure to find bacilli in the lesions, since the degeneration forms, particularly the intracellular fragments, are extremely difficult to identify with certainty in sections. If, however, identical lesions could be produced by the injection of sterile cellular suspensions into the portal circulation, it would exclude the bacterial factor as essential to the production of the liver lesions. To test this possibility, the spleen was removed aseptically from healthy rats, and a rather dense suspension made by teasing the tissue in sterile

Ringer's solution. This was freed from coarser particles, and, after varying periods, injected through a fine glass pipette, into the mesenteric veins of large rats. The injected animals were killed after intervals varying from three and one-half to 48 hours. The results of these experiments were inconclusive. Distinct lesions were found only in the liver of the rat killed after three and one-half hours. In this animal, they simulated so closely the lesions found in the bacillary infection that the suspicion arose that the rat had been spontaneously infected with the disease at the time of the experiment. Cultures, however, yielded only a few colonies of a white staphylococcus. The other four experiments were wholly negative.

This inconclusive result leads us to express with caution a decided view as to the pathogenesis of the lesions. Certain definite inferences may, however, be drawn from a study of the histological features of the disease. It is plain that the bacterial toxin, whatever its nature, brings about an intense destruction of leukocytes and lymphoid cells in all hematopoietic tissues—spleen, lymph-nodes and bone-marrow. Associated with this injury, there is an active proliferation of large mononuclear cells, many of which become phagocytic and in turn degenerate. In many of the organs, and most strikingly in spleen, liver, and lungs, fibrin thrombi are formed about masses of degenerating cells and cell fragments. In the liver where the structural relations are less complicated than in the spleen and bone-marrow, one may state with assurance that the formation of these cell-fragment fibrin thrombi antedates the destruction of the liver cells, and is the first step in the formation of the necrosis. This point, we believe, is clearly brought out by our ink-injection experiments, as well as by a careful study of the early experimental lesions, in which the thrombi may be found unassociated with necrosis of the liver cells.

Whether the liver cells become affected by the mechanical interference with the blood supply, or whether they degenerate because of the toxic effect of bacilli entrapped in the thrombus, or whether possibly they are poisoned by autolytic products from the impacted cells, it is not at present possible to decide.

We wish to express our thanks to Professor W. G. MacCallum and Professor Augustus Wadsworth for suggestions and criticism in the preparation of this paper.

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THE EFFECTS OF EARLY EXTIRPATION OF THE THYMUS IN ALBINO RATS.*

BY ALWIN M. PAPPENHEIMER, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

INTRODUCTION.

Marked differences have been observed in the character and intensity of the changes that follow extirpation of the thymus. These differences may be attributed in part to variations in the conduct of the experiments, but in part they appear to depend upon the particular species of animal used. Most of the recent experimental work has been done upon dogs and rabbits. Fewer, and for the most part poorly controlled observations have been made upon other animals, —goats, guinea pigs, cats, chickens, and frogs,—but they have not led to conclusive results.

The effect of removal of the thymus gland in white rats has not previously been investigated. For this reason, and because it was expected that disturbances in the calcium metabolism resulting from the loss of the gland at an early age might be manifested by changes in the teeth, a systematic study has been made during the past year of the effects of thymectomy upon white rats. The experiments were interrupted last spring by the outbreak of an epidemic, due to infection by a bacillus of the enteritis group, which destroyed many of the rats.[1] The work has been hampered by the sporadic occurrence of this infection, and to a lesser degree by the chronic lung disease which is so often found in rats. As the result of a year's work, therefore, we have only a limited number of uninfected rats from which conclusions as to growth and nutrition may be drawn. However, the experiments have been carefully controlled, and we feel justified in reporting the data, especially since the known nor-

* Received for publication, December 29, 1913.

[1] A study of the bacteriological and pathological findings in this disease will appear in a forthcoming number of the *Journal of Infectious Diseases.*

mal variations in growth and weight, as recently worked out in detail by Hatai (1) and Jackson (2), serve as further controls. Their figures emphasize the need for caution in drawing inferences from slight differences in weight. This is true even for animals of the same litter, maintained under apparently identical conditions.

The literature relating to the experimental extirpation of the thymus has been collected in the exhaustive reviews of Hammar (3), Wiesel (4), Klose (5), Biedl (6), Matti (7), and Hart (8). Since the effect of removing the thymus of rats has not previously been studied, reference will be made only to the observations that bear upon our work.

METHODS AND TECHNIQUE.

The removal of the thymus was performed upon young rats, ranging in weight from ten to twenty-five grams. When possible the age was accurately determined, but in some litters the age had to be estimated. Probably none of the operated animals were over twenty-one days old when the gland was removed. Two or more rats from each litter were reserved as controls, and kept in the same cage as the operated rats.

The operation was performed aseptically under ether anesthesia. A median incision was made in the lower part of the neck and continued over the upper half of the thorax. The sternum was then split as nearly as possible in the median line as far as the fourth or fifth intercostal space, and the edges were retracted. The triangularis sterni muscle was separated, exposing the thymus, which was carefully dislodged from below upwards with fine blunt forceps, and removed when possible, without laceration or separation of the two lobes. The friability of the gland made this the most difficult part of the operation. The sternum and skin were sutured with silk. Healing in almost all cases took place rapidly and without suppuration. It was found advantageous to use a simple positive pressure apparatus, to prevent collapse of the lung when the pleura was accidentally injured, and to institute artificial respiration when necessary.

In the earlier experiments the control rats were also subjected to etherization and the sternum was incised without removal of the

thymus. It was found later that the operation had practically no immediate effects, and this precaution was therefore considered unnecessary.

After the end of the nursing period, the rats were fed principally on bread, sunflower seed, and greens. Meat was not given, and the feeding of milk was given up, since it seemed to favor the spread of infection.

The operated rats together with the controls were kept under observation for varying periods up to six months, and were then killed. They were weighed weekly.

At autopsy the organs of the neck and thorax were removed together. After fixation the lungs and lower half of the heart were dissected off, and the rest of the tissue was embedded in paraffin and cut in series of 10 micra in thickness. In the earlier litters each section was mounted, but later only every tenth section was examined. When there was doubt as to the nature of the tissue, the intermediate sections were also studied.

The other organs were fixed in Zenker's fluid, and stained with hematoxylin and eosin. The bones and teeth were fixed in formalin and decalcified in phloroglucin nitric acid, or in some cases, in Müller's fluid. The adrenals were fixed in Müller-formol solution.

EXPERIMENTAL PART.

26 litters, comprising 118 rats, were used for this study. Of these, 82 were thymectomized, the remainder serving as controls. 19 rats died during or soon after the operation. 5 litters, including 14 operated rats and 11 controls, are still under observation. In 18 of the remaining 49 rats, extirpation was proved to have been complete by serial section through the entire neck organs. In one of the rats accessory thymus tissue was found. 5 of the 18 rats showed lesions characteristic of infection with the bacillus of the enteritis group mentioned above, and are excluded on this account.

The following abbreviated protocols, therefore, refer only to rats in which entirpation was proved to have been complete, in which no accessory tissue was present, and which were shown by microscopic study to be free from infection.

The data given in table I afford a decisive answer to the question

TABLE I.

Summary of Protocols.

Litter.	Sex.	Age at operation.	Weight at operation.	Weight at death.	Duration of life after operation.	Remarks.
A2	F.	14 dys.	14.1 gm.	58.5 gm.	31 dys.	Killed with chloroform.
A3	M.	14 dys.	16.3 gm.	66.0 gm.	31 dys.	Killed with chloroform.
A5	F.	14 dys.	14.6 gm.	62.0 gm.	31 dys.	Control: sternum incised.
A6	F.	14 dys.	14.6 gm.	65.0 gm.	31 dys.	Control.
B2	M.	15 dys.	16.1 gm.	86.9 gm.	43 dys.	Killed with chloroform.
B6	M.	15 dys.	16.4 gm.	98.6 gm.	43 dys.	Control: sternum incised.
E2	M.	10 dys.	12.0 gm.	77.5 gm.	58 dys.	Killed with chloroform.
E6	F.	10 dys.	12.0 gm.	59.0 gm.	58 dys.	Control.
F1	F.	10 dys.(?)	18.5 gm.	70.0 gm.	99 dys.	Killed with chloroform.
F4	F.	10 dys.	18.0 gm.	111.0 gm.	99 dys.	Control (infected).
M3	F.	13 dys.	11.0 gm.	33.0 gm.	70 dys.	Spleen removed 11 days after thymectomy.
M7	F.	13 dys.	11.0 gm.	49.0 gm.	82 dys.	Control: spleen removed at same time as M3.
O1	M.	20 dys.	21.0 gm.	107.0 gm.	120 dys.	Killed with chloroform.
O2	M.	20 dys.	21.5 gm.	106.0 gm.	120 dys.	Killed with chloroform.
O3	F.	20 dys.	23.0 gm.	87.0 gm.	131 dys.	Killed with chloroform.
O5	M.	20 dys.	23.5 gm.	122.5 gm.	120 dys.	Control.
O6	F.	20 dys.	21.5 gm.	83.0 gm.	131 dys.	Control.
P1	F.	23 dys.	24.5 gm.	137.0 gm.	130 dys.	Killed with chloroform.
P2	F.	23 dys.	24.5 gm.	111.0 gm.	130 dys.	Killed with chloroform.
P3	F.	23 dys.	24.5 gm.	118.0 gm.	130 dys.	Control.
S2	F.	14 dys.	17.0 gm.	63.0 gm.	95 dys.	Minute thymus rest (?).
S6	M.	14 dys.	18.0 gm.	72.0 gm.	90 dys.	Control.

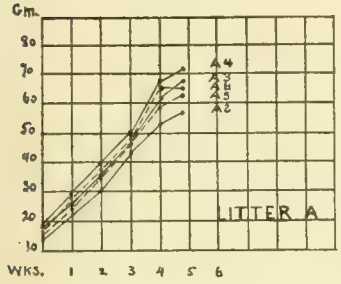
whether the thymus in rats is a vital organ. The period of survival after operation ranged from 45 to 131 days. The animals of the last group (litters O and P) showed no evidence of illness at that time. Among the litters at present under observation are two rats that have survived the operation for 170 days and are still in good health.

It is therefore proved that whatever influence the thymus may have upon growth and nutrition in the rat, it is not essential to life. Sexual maturity, according to Slonaker, is attained at ten weeks, although the maximum weight is not reached until after the first year. The period of observation is therefore sufficiently prolonged, in the case of litters E, M, F, S, O, and P, to carry the animals well past the onset of puberty, at which time in rats (Jackson) and in

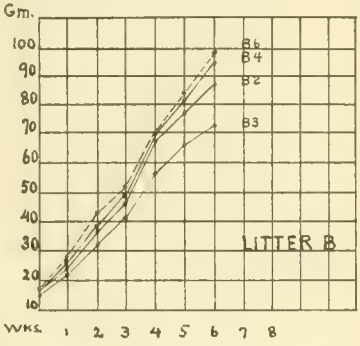
rabbits (Söderlund and Backman (9)), the thymus has begun its normal involution, and is probably no longer at the height of its functional activity. It is not reasonable to suppose that the fatal effects of thymus deprivation would appear only after the anatomic regression of the organ was well under way.

INFLUENCE OF THYMECTOMY UPON GROWTH AND NUTRITION.

An analysis of the weight curves (text-figures 1 to 8)[2] does not indicate a definite influence of thymectomy upon growth.

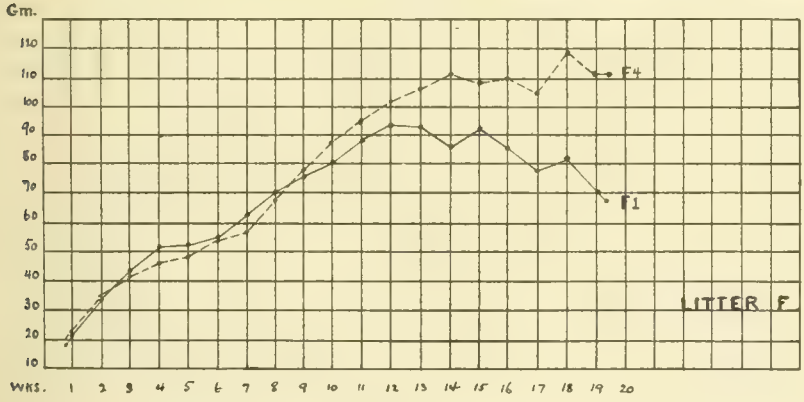


TEXT-FIG. 1.



TEXT-FIG. 2.

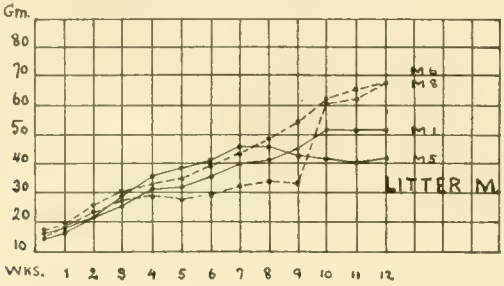
The ten completely thymectomized rats in which microscopic examination showed no evidence of infection were A2, A3, B2, E2, F1, O1, O2, O3, P1, and P2. Two of the rats, A2 and B2, showed



TEXT-FIG. 3.

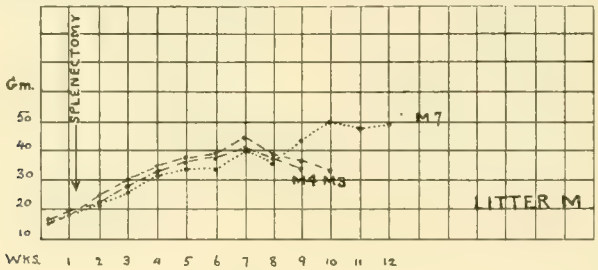
[2] In the charts the unbroken lines represent operated animals, the broken lines controls.

a slight retardation of growth as compared with healthy controls of the same litter. The differences in weight at the time when the



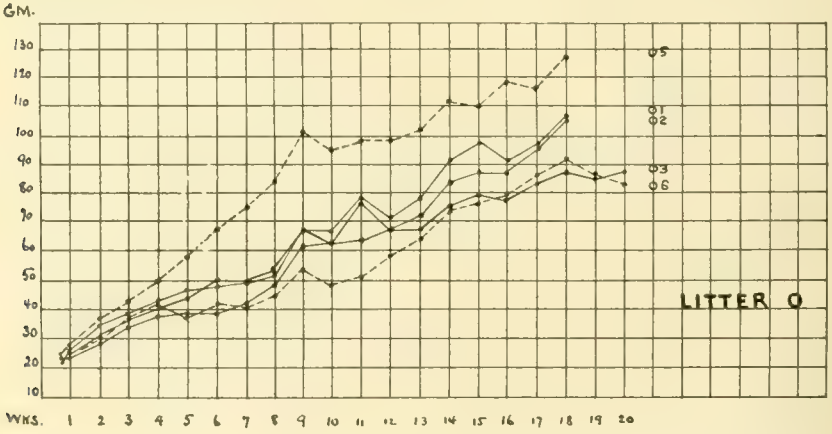
TEXT-FIG. 4.

animals were killed were insignificant and fall easily within the range of normal litter variation as determined by Jackson. Rat



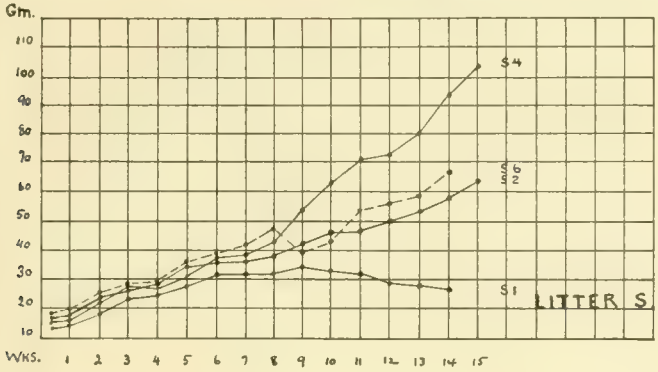
TEXT-FIG. 5.

A3 exceeded both controls. Rats O1, O2, and O3 were midway



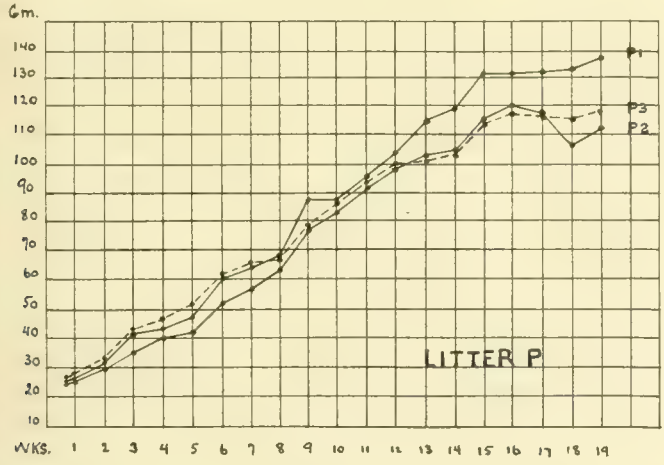
TEXT-FIG. 6.

between the two controls in weight. Rat P1 was above, Rat P2 below the control. Rat F1, ninety-nine days after thymectomy,



TEXT-FIG. 7.

weighed forty grams less than the normal control, F4, but even extreme variations such as this are apparently not uncommon in rats raised under laboratory conditions.[3]



TEXT-FIG. 8.

On the basis of these facts, and taking into consideration our data relating to rats in which small lobules of thymic tissue were

[3] Jackson, C. M., *Am. Jour. Anat.*, 1913, xv, 1. Compare table I, litter M7, in which the weights ranged at 44 days from 23.5 to 71.8 gm. The variations may of course be due to unrecognized pathological conditions.

found, but in which there must have been a marked functional deficiency, one must conclude that removal of the thymus in rats does not noticeably affect growth and nutrition.

BEHAVIOR OF THE OPERATED RATS.

If we leave out of consideration all symptoms that could be attributed to accidental infection and that could be duplicated in normal rats experimentally infected with *Bacillus enteritidis,* no peculiarities in behavior were noted. Particularly to be emphasized is the absence of nervous irritability, tremors, convulsions, and stupor. There was not the slightest evidence of mental deterioration in any of the operated animals, nor was there anything peculiar in their appearance or posture.

LOCAL CHANGES.

The large space left by the removal of the gland becomes replaced by adipose tissue, which for a long time, preserves the character of embryonic fat lobules.

The regional lymphatic glands in the operated rats show a marked hyperplasia. This is accompanied frequently by a distension of the lymph sinuses, with exfoliation of endothelial cells. In some rats the sinuses were found filled with red blood cells, many enclosed in phagocytes.

As regards the behavior of small fragments of thymus tissue not removed at operation, only a few general statements can be made, as a study of the minute regenerative changes do not fall within the scope of the work. The general capacity of small fragments of thymic tissue to regenerate did not appear to be so marked as the statements made by Klose and others led me to believe. The thymic rests found in serial sections were often extremely minute and showed no evidence of active proliferation. They were composed of densely packed small cells, among which were a few complexes of larger, paler epithelial cells. Fully formed, concentrically disposed Hassall bodies were only exceptionally seen. The identification of small masses of tissue as thymus was therefore somewhat difficult, and rested upon the absence of peripheral lymph sinus and the presence of the epithelial cell complexes. Because of the closer crowding of the cells, the staining of the thymus tissue was somewhat deeper than that of the adjacent lymph nodes.

A further differential point observed was the absence of necrotic lesions in the thymic tissue in rats infected with *Bacillus enteritidis.* The surrounding lymphoid tissue in these rats regularly contained areas of focal necrosis, but in not a single instance was the thymus or the rest of thymic tissue similarly affected. This is in accord with the well known insusceptibility of the thymus to tuberculosis and other specific infections.

THE INFLUENCE OF THYMECTOMY UPON OTHER ORGANS.

Skeletal System.—Particular attention was given to possible alterations in the bones and teeth. The femur, tibia, upper and lower incisors, and in some cases, the ribs, were examined. The methods used included partial decalcification in Müller's fluid, and staining with hematoxylin and carmine after Schmorl.

I have failed to observe any characteristic qualitative changes which might be brought into relation with the operation. Whenever marked retardation of growth occurred, the bones were shorter, the epiphyseal cartilage narrower, the cortical and trabeculæ more slender, and the marrow spaces larger than in the controls. In not a single instance, however, was there marked gross deformity, nor was there any irregularity in the processes of ossification. Rachitis-like lesions like those described by Klose (10) and Matti (11) in dogs, and by Soli (12) in rabbits were wanting.

The teeth also showed no irregularities in the calcification of the dentine, and none of the animals that were observed over a long period showed spontaneous infarctions such as have been described by Erdheim (13) and Toyofuku (14) in parathyroidectomized rats.

In the absence of chemical studies it seems unwarranted to deny the influence of the thymus in rats upon skeletal and dental calcification. The foregoing observations, however, certainly afford no histological evidence pointing to such an influence.

Adrenals.—There has been brought forward considerable pathological and experimental evidence indicating a correlation or antagonism between the thymus and the adrenals. Matti (11) states that in thymectomized dogs, there was regularly observed, on macroscopic examination, a hypertrophy of the adrenal medulla. Histologically, there were found the changes described by Stoerk

and von Haberer (15), as characteristic of hypertrophic medullary tissue. Other references to the condition of the adrenals after thymectomy are less definite. Soli found the adrenals diminished in weight in six chickens and increased in five; in eight guinea pigs it was increased in six and diminished in two. Nordmann (16) describes a hyperemia. Lucien and Parisot (17) found a slight diminution in the absolute weight, and no appreciable change in the relative weight of the adrenals after thymectomy.

Hyperplasia of the thymus after adrenalectomy is described by Calogero (18), Bonnet (19), and Auld (20), and, as pointed out by Hedinger (21), Hart (22), Wiesel (23), Pappenheimer (24), and others, occurs frequently in Addison's disease.

I have not found definite alterations in the adrenals in thymectomized rats, either in weight or in histological structure. The chromaffin staining tissue was invariably abundant, but not more so than in the controls. Changes in the chromaffin cells which could be interpreted as indicating a hypertrophy were not found. The cortex also showed no structural difference from that of the control ad-

TABLE II.

Weight of Adrenal Glands.

Rat.	Sex.	Operation.	Absolute weight.	Relative weight.
M1	F.	Thymectomy (I)[4]	0.030 gm.	1–1733
M5	F.	Thymectomy (C)[4]	0.020 gm.	1–2100
M6	M.	Control	0.020 gm.	1–3350
M7	F.	Splenectomy	0.020 gm.	1 2450
M8	M.	Control	0.011 gm.	1–6909
O1	M.	Thymectomy (C)	0.022 gm.	1–4818
O2	M.	Thymectomy (C)	0.024 gm.	1–4396
O3	F.	Thymectomy (C)	0.017 gm.	1–5118
O4	M.	Control	0.024 gm.	1–5083
O5	F.	Control	0.021 gm.	1–3952
P1	F.	Thymectomy (C)	0.030 gm.	1–4560
P2	F.	Thymectomy (C)	0.025 gm.	1–4432
P3	F.	Control	0.028 gm.	1–4214
S1	F.	Thymectomy (C)	0.013 gm.	1–2083
S2	F.	Thymectomy (C?)	0.021 gm.	1–3000
S4	M.	Thymectomy (I)	0.027 gm.	1–3233
S5	F.	Splenectomy	0.015 gm.	1–3233
S6	M.	Control	0.020 gm.	1–3600
U4	M.	Thymectomy (I)	0.020 gm.	1–1800
U7	M.	Control	0.017 gm.	1–2200

[4] In the tables, I = incomplete, and C = complete.

renals, although special staining of the lipoids was not attempted. The absolute and relative weights of the adrenals in some of the operated litters are appended.

Although at first sight the figures appear to indicate a relative increase in the weight of adrenals in the thymectomized rats, this is probably due to the relatively small weight of many of the operated animals rather than to an hypertrophy. That it cannot be directly attributed to the loss of thymus is indicated by the fact that the weight is relatively high also in the splenectomized rats, M7 and S5. The highest relative weights, moreover, are found in rats M1 and U4, which were infected and cachectic, and in which the extirpation of the thymus was incomplete.

Testes.—The condition of the testes in the operated animals and in the corresponding controls is given in table III.

TABLE III.

Testes.

Rat.	Age.	Operation.	Weight of testicles.	Size of testicles.	Spermatogenesis.
A3	45 dys.	Thymectomy		4.5 mm.	No spermatogenesis.
A4	45 dys.	Thymectomy (I)		12.0 mm.	No spermatogenesis.
A6	45 dys.	Control		6.5 mm.	No spermatogenesis. More mitotic figures than A3 and A4.
B2	58 dys.	Thymectomy (C)	1.51 gm.		Spermatogenesis.
B4	58 dys.	Thymectomy (I)	1.45 gm.		Spermatogenesis.
B6	58 dys.	Control	1.50 gm.		Spermatogenesis.
E2	68 dys.	Thymectomy (C)		16.0 mm.	Active spermatogenesis.
E3	68 dys.	Thymectomy (I)		11.0 mm.	No spermatogenesis.
O1	140 dys.	Thymectomy (C)	1.456 gm.		Active spermatogenesis.
O2	140 dys.	Thymectomy (C)	1.425 gm.		Active spermatogenesis.
O5	140 dys.	Control	1.664 gm.		Active spermatogenesis.
S4	104 dys.	Thymectomy (I)	1.130 gm.		Fairly active spermatogenesis.
S6	104 dys.	Control	0.605 gm.		Fairly active spermatogenesis.
U4	79 dys.	Thymectomy (I)	0.180 gm.		No spermatogenesis.
U7	79 dys.	Control	0.296 gm.		No spermatogenesis.

In litter A spermatogenesis was not fully established at forty-five days, when the rats were killed. In litter B, at fifty-eight days, both thymectomized and control rats showed spermatogenesis; and this

was true in all the other testes examined, except in litter E. Here the development of the testis was further advanced in E2 than in another male of the same litter, E3, in which the removal of the thymus was incomplete. The data are not sufficiently numerous to warrant a final conclusion, but as far as they go, they do not indicate a decided effect of thymectomy upon the testes.

The literature bearing on this question contains contradictory statements. The most extensive experiments made to determine the influence of thymectomy upon the development of the testes are those of Paton (25). In guinea pigs removal of the thymus was followed by increase in the average weight of the testes, amounting in one series to 46 per cent. Microscopic examinations were not made.

In chickens, rabbits, and guinea pigs Soli (26) obtained opposite results. The testicles in the thymectomized animals killed after five months, were smaller, weighed less than the controls, and failed to show active spermatogenesis. Lucien and Parisot also found a transient retardation in the development of the testicles.

Klose and Vogt (10) substantiated in dogs the findings of Paton in that they observed a transient hyperplasia of the testes, which disappeared in the cachectic stage. Matti (11), on the other hand, was unable to determine any relation between thymectomy and the onset of spermatogenesis.

In view of these conflicting statements and our negative findings in rats, it seems premature to attribute to the thymus a direct influence upon the male genital glands. That there exists an inverse relation, however, by which the involution of the thymus is retarded after early castration, may be considered proved, and the numerous experiments of Henderson (27), Goodall (28), Calzolari (29), Ranzi and Tandler (30), and Soli and Gellin (31) are in agreement on this subject.

Thyroid.—In many of the operated animals the thyroid showed a slight variation in structure from that of the controls. The acini were smaller, the epithelium higher, and the colloid less abundant and less densely stained than in the controls. These differences, however, were not striking, and marked epithelial hyperplasia, with papillary infolding of the epithelium, such as Matti (11) describes

in thymectomized dogs, was not seen. The impression gained was that the alterations in the thyroid were not specific, but merely accompanied the arrested development. They were equally manifest in rats whose development was retarded by infections or other causes.

Spleen.—Soli found the weight of the spleen in chickens and rabbits unaffected by thymectomy. Lucien and Parisot also found no changes in the weight, but observed in certain cases an increase in the number and size of the follicles. Perrier (32), Klose and Vogt, and Matti have also observed a follicular hyperplasia, followed by atrophy in the athreptic stage.

Klose (5) concludes from these facts that the spleen "may to a certain extent and after a certain interval, take over the function of the ablated thymus." Late extirpation of the thymus, which ordinarily causes only transitory "rachitis-like disturbances of growth," if combined with splenectomy, leads to death within a few weeks. Matti (7) disputes both the facts and the inferences that Klose draws.

I have repeated this experiment in three rats, M3, M4, and S3. In M3 and M4, the spleen was extirpated 11 days after thymectomy. The animals gained steadily in weight until the 47th day after thymectomy, when the weight curve began to decline. M4 was found dead and partially eaten on the 66th day after operation. M3 became emaciated and weak, and developed diarrhea. It was killed on the 70th day, and extirpation was proved by serial section to have been complete. A control rat, M7, in which the spleen alone had been removed, weighed at this time seventeen grams more than M3, but also showed a retardation of growth as compared with the unoperated controls, M6 and M8.

This experiment proves that a rat may live for at least fifty-nine days after extirpation of both thymus and spleen. Since the microscopic examination showed ulcerative lesions in the intestine and inflammatory foci in the lungs, it is probable that death was due to intercurrent infection rather than to the lack of these organs.

In rat S3, which was splenectomized fifteen days after removal of the thymus, there was a normal gain in weight surpassing that of a control rat of the same litter (S5) in which the spleen alone had

been extirpated, but lagging somewhat behind the two unoperated controls. It was found dead and partially eaten eighty-eight days after thymectomy. Since the neck organs could not be examined serially, it was not possible to be sure that the thymus had been completely removed.

The absolute and relative weights of the spleen in our series of animals are given in table IV. There is wide variation in the weights

TABLE IV.

Weight of Spleens.

Operated rats.		Controls.	
A2	0.25 gm.		
B2	0.62 gm.	B6	0.325 gm.
B3	0.22 gm.		
M1	0.19 gm.	M6	0.205 gm.
M5	0.19 gm.	M8	0.285 gm.
O1	0.397 gm.		
O2	0.301 gm.	O5	0.396 gm.
O3	0.270 gm.	O6	0.238 gm.
P1	0.357 gm.		
P2	0.271 gm.	P3	0.292 gm.
S1	0.192 gm.		
S2	0.425 gm.		
S3	0.818 gm.	S6	0.560 gm.
U4	0.220 gm.	U7	0.166 gm.

and those of the thymectomized rats are sometimes heavier and sometimes lighter than those of the controls. The same variation was noted in the histological pictures. The size of the follicles in well nourished rats was large; in athreptic animals an atrophy of the lymphoid tissue occurred regularly. In no case where the nutrition of operated and control rats was equally good, were striking differences found in the histological picture. It seems therefore that thymectomy in rats does not lead to constant or definite alterations in the structure of the spleen.

Blood.—The differential blood counts[5] on four operated litters are given in table V. The counts are not sufficiently numerous to

[5] I wish to express my gratitude to Miss Gertrude Fisher for making the blood counts. Although the counts were not continued for a longer period than five weeks after the operation, the data are given since they may prove of use to other workers in this field.

TABLE V.

Litter M. Differential Count Made on August 14, 1913, Five and One Half Weeks after Thymectomy.

		Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Eosinophils.
M1	Incomplete thymectomy; infection	36	26	22	8	8	0
M2	Complete thymectomy and splenectomy; no infection	46	18	22	10	4	0
M3	Complete thymectomy and splenectomy; no infection	21	38	25	8	8	0
M4	Complete thymectomy	28	29	20	11	12	0
M5	Control	30	16	43	7	4	0
M6	Control: splenectomy	30	27	30	13	5	0
M7	Control	35	19	24	17	5	0

Litter S. Operated July 22, 1913.

S1	Complete thymectomy						
	July 24	17	56	22	12	3	0
	July 31	41	16	17	15	11	0
	Aug. 9	41	31	16	11	1	0
	Aug. 14	34	32	24	3	7	0
	Aug. 21	36	26	25	8	5	0
S2	Complete(?) thymectomy						
	July 24	23	26	30	7	4	0
	July 31	39	29	22	5	5	0
	Aug. 9	35	18	31	13	3	0
	Aug. 14	30	21	27	14	8	0
	Aug. 21	31	15	32	14	8	0
S3	Thymectomy, splenectomy (Aug. 6)						
	July 24	17	45	24	9	4	0
	July 31	42	20	26	8	4	0
	Aug. 9	33	27	28	4	8	0
	Aug. 14	46	34	9	6	5	0
	Aug. 21	43	8	17	18	14	0
S4	Control: splenectomy						
	July 24	13	60	20	4	3	0
	July 31	11	76	8	2	3	0
	Aug. 9	15	61	17	4	3	0
	Aug. 14	44	18	22	11	5	0
	Aug. 21	22	46	21	7	4	0

TABLE V.—*Concluded.*

		Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Eosinophils
S5	Control: no infection						
	July 24	14	68	13	1	4	0
	July 31	8	58	30	1	3	0
	Aug. 9	16	53	18	6	2	0
	Aug. 14	15	46	20	12	7	0
	Aug. 21	25	25	35	7	8	0
	Litter W.[6] *Operated November 22, 1913. Still under Observation.*						
W1	Thymectomy						
	Oct. 27	46	14	14	26[7]		0
	Nov. 6	28	39	22	11		0
	Nov. 12	42	24	26	8		0
	Nov. 17	40	32	20	8		0
	Nov. 25	50	25	29	6		0
W2	Thymectomy						
	Oct. 27	33	48	10	9		0
	Nov. 9	35	54	3	8		0
	Nov. 12	43	27	31	9		0
	Nov. 17	48	14	27	9		2
	Nov. 25	32	15	46	7		0
W3	Thymectomy						
	Oct. 27	60	18	14	8		0
	Nov. 1	58	20	16	6		0
	Nov. 12	43	21	31	5		0
	Nov. 17	49	15	31	5		0
	Nov. 25	49	10	34	8		0
W4	Control	22	62	9	7		0
		13	46	36	5		0
		28	35	14	14		0
		37	20	29	14		0
		44	32	18	6		0
W5	Control	13	52	26	9		0
		11	63	19	7		0
		44	25	24	7		0
		23	40	33	4		0
		40	25	27	4		4

warrant a minute analysis or to permit of generalizations. There is an evident tendency, particularly in litter S, towards the occurrence of a relatively high proportion of polymorphonuclears during the first weeks after operation. Since the wounds healed aseptically

[6] The total leucocyte count in this litter showed no differences between operated rats and controls. Leucocyte counts were also made on the rats of litters S and P, and no variation from the normal was found.

[7] Large mononuclears and transitionals were counted together.

and the polynucleosis persisted after complete healing, the change in the blood picture is probably due to the loss of the thymus rather than to the effect of the operation.

Previous studies of the blood changes in other animals following thymectomy are contradictory. Seiler (33), who made blood counts upon one of Matti's operated litters of dogs, found a delay in the establishment of the normal percentage of polynuclears; the lymphocytic count remained high for a longer period than in the controls. Matti attributes this to the general retardation in development. However, an inspection of the actual counts made by Seiler several months after the operation shows that the percentage of polymorphonuclears in the thymectomized dogs was slightly higher (72 to 81 per cent.) than in the two controls (65 to 68 per cent.).

Lampé and Liesegang (34), who carried out blood studies on Vogt's animals, found a progressive diminution of the lymphocytes to far below their normal values. This is more in accord with my findings, and is also in harmony with the well known occurrence of relative lymphocytosis in Status lymphaticus, Basedow's disease, and other alterations of the ductless glands commonly associated with hyperplasia of the thymus.

CONCLUSIONS.

It has been shown that white rats may survive complete extirpation of the thymus for at least 131 days, even when the operation is performed within the first two weeks of life.

Removal of the thymus does not produce an arrest or retardation of body growth and development.

Qualitative changes in the skeletal system or teeth have not been found. In emaciated, weak animals osteogenesis is less active than in healthy rats, and the long bones are smaller and more delicate in structure. Such quantitative differences appear to depend upon the general nutrition, are equally pronounced in rats whose development is retarded from other causes, and cannot be referred specifically to loss of thymus function.

No constant or characteristic alterations were detected in the spleen, testes, adrenals, or thyroid. Whatever functional correla-

tions may exist between thymus and any or all of these organs are not evident from the occurrence of histological changes after the removal of the thymus.

The relative proportion of lymphocytes in the blood is diminished for the first few weeks after the operation. We have not determined how long this alteration in the leucocytic formula persists.

Since this paper went to press an article has appeared by Klose,[3] describing briefly the results of thymus extirpation in pigs, goats, rats, and chickens. In rats thymectomized on the fourteenth day, there followed a progressive cachexia terminating in death after eight to ten weeks. Disturbances in ossification, which macroscopically and microscopically were identical with those of human rickets, developed also in the ribs and long bones. Some of Klose's litters failed to show these lesions, and this negative result is explained as having been due to the presence of thymic tissue within the thyroid gland.

The observations of Klose are in direct contradiction to the negative results described in this paper. Since the possible presence of accessory thymus tissue either within the thyroid or elsewhere was carefully excluded in my experiments, the discrepancy between my findings and those of Klose cannot be explained upon this basis.

Since this paper was sent to the publisher I have studied two additional rats which were killed 185 days after operation. Minute examination of a complete series of the neck organs, including the thyroid, failed to show any tissue which could be interpreted as thymus. The bones showed no rachitic changes.

The infective origin of rachitic and osteomalacic lesions in rats has been established by Morpurgo. Although reference is made by Klose to Morpurgo's work, the data given by him do not enable one to judge whether this infection was definitely excluded.

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COMPARATIVE STUDIES UPON CANCER CELLS AND NORMAL CELLS.

II. The Character of Growth in Vitro with Special Reference to Cell Division.*

By ROBERT A. LAMBERT, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

Plates 98 to 100.

In presenting this report it is realized that general conclusions upon the nature of cancer based upon the observation of cells cultivated under artificial conditions should be formulated with hesitation. It may be pointed out, however, that our previous studies, as well as a part of the present investigation, were undertaken to show the similarity in the behavior of tumor cells *in vitro* and *in vivo*.

A series of comparative studies upon cancer cells and normal cells have been made in this laboratory during the past year. The first report upon this work dealt with the susceptibility to heat of sarcoma cells and connective tissue cells (1). The present report will be limited to a consideration of the general character of growth *in vitro* of the same two tissues with special reference to cell division.

GENERAL CHARACTER OF GROWTH.

Recent papers on the subject render unnecessary a detailed description of the general phenomena of growth in tissue cultures and the technique of the method. We have used two transplantable tumors as material for growing malignant tumor cells; one a spindle cell sarcoma of the rat, the other a mixed cell sarcoma of the mouse. Normal connective tissue cells were grown chiefly from small pieces of blood vessel removed from growing or adult animals.

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Primary cultures of cells from the two kinds of sarcoma and the blood vessel differ rather strikingly, though not fundamentally, as a brief description of each will show. A piece of blood vessel placed in a hanging drop of plasma shows at the end of twenty-four hours few if any cell outgrowths and forty-eight hours may elapse before activity is noted; but at the end of the third or fourth day a fairly dense halo of cells is seen surrounding the tissue fragment. Growth, with slow radial extension of the cells into the coagulum, may continue for several days longer. The cells are for the most part spindle in form, with long, delicate processes at either extremity. There may be, however, considerable variation in morphology, the size and shape of the cells depending largely on their position in the hanging drop: those creeping along the cover-glass or upon the surface of the coagulum may be flattened and polygonal in outline, while those wandering through the clot are more compact, spindle-shaped, triangular, or radiate in form, and possess long processes.[1] Karyokinetic figures are occasionally seen. The accumulation of fat in the cells proceeds slowly reaching a maximum after five or six days.

Although the sequence of changes in sarcoma cultures is much the same as in cultures of blood vessel, the rate of their occurrence is more rapid. There is practically no latent period; and cells begin to migrate within a few hours. At the end of twenty-four hours the culture has often reached the height of its activity and numerous mitotic figures are seen (figure 1). By the end of the second day many of the actively migrating cells have reached the edge of the drop of plasma. The cells have become loaded with fat, and growth may cease unless the tissue is transferred to fresh culture media.

It is thus seen that in primary cultures the differences in the general character of growth of sarcoma and connective tissue are such as might have been anticipated, being, apart from the morphol-

[1] Failure to recognize the influence of mechanical factors upon the morphology of cells growing in cultures may easily lead to erroneous interpretations; several types of tissue cells may, for example, be described in a preparation in which only one kind of cell is growing. This source of error is illustrated by figure 13 in a recent paper by Lambert and Hanes (Lambert, R. A., and Hanes, F. M., *Virchows Arch. f. path. Anat., loc. cit.*, p. 106.)

ogy of the cells, chiefly in the rate and extent of growth and ameboid wandering.

In secondary and subsequent cultures differences in rate of activity are likewise noticeable, but the relations are considerably altered. Sarcoma cells grow less vigorously, cell wandering and multiplication being less marked than before. In the case of mouse sarcoma we have found it difficult to propagate the cells through subcultures for any length of time. Connective tissue cells, on the contrary, grow more vigorously than in primary cultures. The cells begin to wander out almost immediately. Mitotic figures are often extremely numerous (figure 2). In one preparation more than a hundred cells were observed undergoing mitotic division simultaneously. Apparently the resting connective tissue cells of the vessel wall have become transformed within a few days into actively proliferating fibroblasts, similar to what occurs during the reparative process following a wound in the body. It is conceivable that the mechanism of the reaction is the same in both cases.

That secondary cultures of connective tissue cells are more regularly successful, exhibit many more mitoses, and often show a more extensive growth than secondary cultures of sarcoma, has been shown conclusively in a large number of experiments. Several explanations of this fact, each of which upon analysis seems inadequate, have suggested themselves: (1) that tumor cells are in general less hardy than normal cells and the failure to thrive under artificial conditions is but an evidence of a hypothetical lowered vitality; (2) that tumor tissue requires a larger food supply than normal connective tissue and the food supply in tissue cultures is insufficient for the continuous active growth of sarcoma cells; (3) that some substance, as, for example, the internal secretion of one or more glands, upon which the continuous multiplication of tumor cells may depend, is present in insufficient amount or is too irregularly supplied in cultures.

The first explanation, which postulates a low resistance to injuries of all kinds on the part of tumor cells, has some support. Ribbert (2) some years ago expressed this view, basing his opinion on the clinical observation of the effects of radium, x-ray, etc., which exhibit a more destructive action upon cancer cells than upon

the adjacent normal tissues, and he suggested that the low vitality of the cells was caused by a poor circulation. In a recent paper (1) we described experiments showing that rat sarcoma cells are less resistant to heat than are normal connective tissue cells. On the other hand, the vitality exhibited by the cells of rat and mouse tumors under different conditions of transplantation indicates at least a rather high degree of resistance to mechanical injuries. Furthermore, the greater resistance to low temperatures of mouse tumor cells, as compared with normal body cells, can hardly be questioned.

The second explanation involves the assumption that tumor tissue requires more food than an equal quantity of normal tissue. Experimental data on this point are meager: such as we have, however, do not support the notion. Cramer and Pringle (3), for example, have shown that in the case of a rat sarcoma less nitrogen is required to build up a certain amount of tumor than to build up an equal weight of somatic tissue.

Our knowledge at present of the factors controlling the growth of normal tissues is so limited that the general idea contained in the third explanation, namely, that the unlimited growth of tumor cells is dependent upon some body secretion which is insufficiently supplied in cultures, can hardly be discussed. While there is a general tendency to attribute to tumor cells an independence of body function, such an independence has not been proven.

It was mentioned in a former paper that we had been able, by successive transfers, to propagate rat connective tissue cells *in vitro* for more than three months, at which time the cells were still multiplying actively. No changes indicating degeneration from old age were observed. While this experiment covers too short a period to be of much significance, it suggests a reference to the point of the immortality, so called, of the cancer cell which the cancer transplantation experiments of the past twelve years tend to support. The fact that tumors arising in rats and mice have been propagated by successive transplantation to other hosts over a period several times that representing the life of the animal in which the tumor originated, has been emphasized. It will be interesting to see if we shall be able to cultivate normal cells (of the mouse, for

example) for a period exceeding the life of an individual of that species. It may thus be possible to determine whether or not the attribute of immortality is to be regarded as characteristic of the cancer cell.

CELL DIVISION.

The presence of atypical division figures in the cells of malignant new growths has been noted by a number of observers (Cornil, Klebs, von Hansemann (4), Galeotti (5), Farmer, Moore, and Walker (6), Bashford and Murray (7), and others.[2] The contributions of most interest, on account of the theoretical importance attached to the observations recorded, are those of von Hansemann, and Farmer, Moore, and Walker.

von Hansemann regarded the presence of atypical mitoses in tumors as an expression of a change in biological character that occurs when normal cells become anaplastic, or undifferentiated. It is the anaplastic cell, he maintains, which, upon receiving the proper stimulus, becomes the cancer cell.

Farmer, Moore, and Walker, who saw in the abnormal mitoses in tumors a striking resemblance to the type of division in reproductive tissue (heterotypical mitosis), attached another significance to the phenomenon. Their theory is as follows: The remote cause of cancer, which may be some form of chronic irritation, induces in normal cells a change in their mode of division which results eventually in a definite heterotypical karyokinesis. Cells exhibiting this type of division possess the inherent power of unlimited growth. The mechanism, then, of the development of cancer cells, consists essentially in the production of a change, through some unknown agency in the character of division in normal body cells. This theory invites attack first on account of the assumption that reproductive cells possess a power of unlimited infiltrative growth. In support of this assumption they refer to the parasitic growth assumed by the cells of the embryo sac in certain flowering plants. von Hansemann has criticized this example on the ground that such a parasitic growth bears no analogy to the invasive growth of malignant tumor cells in the body. It is sufficient to point out, as Bashford says, that there is no evidence either in histological studies or in experimental work, such as transplantation of testis and ovary or ligation of the vas deferens, that the reproductive cells of vertebrates possess the properties postulated in this theory.

The question as to whether the mitoses in cancer are definitely heterotypical, as Farmer, Moore, and Walker maintain, will be taken up later.

The work upon which we shall report at this time is concerned with a study of mitosis in stained and fresh cells in cultures of

[2] An exhaustive review of the subject with a complete bibliography is given by Wolff, J., Die Lehre von der Krebskrankheit von den altesten Zeiten bis zur Gegenwart, Jena, 1907, i.

sarcoma and connective tissue. The method used permits of a more complete study of the process of cell division in vertebrate tissues than has hitherto been possible, since observations can be made upon living growing cells (figure 6). Through such observations serial pictures made from the same cell can be obtained, and the time required for division, which, as we shall show, differs markedly in normal and cancer cells, can be determined. The method also permits one to fix and stain a mitotic figure in any phase, thus making it possible to verify observations upon the living structure (figure 7). For this reason alone the method will appeal to those interested in a finer study of normal and pathological mitoses in the tissues of higher animals. It should be explained that with this method sections of the tissue and media are not necessary, and the cells are usually well separated in the plasma and are often so flattened against the cover-glass or on the surface of the coagulum that a determination of the number of chromosomes is no more difficult than in paraffin sections (figure 3, *c*).

The questions investigated have been (1) the character of the mitoses, and (2) the rate of division in sarcoma and connective tissue cells.

CHARACTER OF THE MITOSES.

According to von Hansemann (8) and others, atypical division figures in cancer cells may be of three kinds: (1) asymmetrical mitoses; (2) multipolar mitoses; and (3) hyper- and hypochromatic cells. Asymmetrical mitoses were regarded by von Hansemann as of most significance; they were found only in carcinoma. Distinct asymmetrical figures have not been found in our sarcoma preparations. Multipolar mitoses, however, were not infrequently encountered. A tripolar mitosis is shown in figure 5, *b*. That multipolar division of the nucleus may result in the formation of multinucleated cells, appears probable from observations to which reference will be made.

Abnormal pictures suggesting tetrapolar mitoses were occasionally noted in fresh cells but upon fixing and staining the cells it was seen that a lagging of chromosomes was responsible for the appearance. The cell in figure 4, *b*, and probably also the one in

figure 5, *a*, are instances of this. The cell in figure 4, *b*, had been under microscopical observation in the warm box for about thirty minutes; the chromosomes were distinctly visible in the equatorial plate stage. The mass of chromosomes began to break up but instead of a division into two equal groups with segregation at the poles of the cell, there seemed to be a general scattering. After a few minutes two irregular clumps could be made out situated near the poles; the remaining chromatin material could be seen with difficulty. The preparation was then fixed with formalin vapor and stained with iron hematoxylin.

Hyper- and hypochromatic mitoses were somewhat more common than multipolar figures (figures 1 and 4, *a*). Hyperchromatic cells seemed to be more numerous than cells with a diminished number of chromosomes. A large number of counts, however, was not made, since a study of this kind did not appear necessary in view of the studies of von Hansemann and his pupils, and the recent work of Bashford and Murray (9).

Their results indicate, in brief, that while a reduction in the number of chromosomes is often observed in cancer cells, the reduction is not an exact halving; furthermore, the chromosomes are not of the same character as those found in gametogenic cells. An analysis of Farmer, Moore, and Walker's papers shows that according to their own observations only a small percentage of the mitoses in cancer are definitely heterotypical. This fact in itself should at least lessen the force of their conclusions.

von Hansemann maintained that hypochromatic cells were developed in two ways: first, by asymmetrical division, and secondly, by a casting out and degeneration of chromosomes during division. The latter phenomenon we have observed in unstained cells. In the instances noted, one or more chromosomes were seen to be left behind as the two masses of chromatin separated in anaphase. The lost chromosomes could be followed long enough for the observer to be convinced that they did not enter into the formation of the daughter nuclei; but their ultimate fate was not determined with certainty. They appeared to become dissolved in the cell cytoplasm.

Amitotic division has been described as a fairly common occurrence in tumor cells and as taking place not infrequently in connective tissue cells. It seemed, therefore, of interest to search for evidences of the process in living cells. The ease with which mitosis may be studied in unstained preparations made it appear probable that stages in amitotic division, if present, might also be followed. However, after observations upon several hundred cells

undergoing division, an example of amitosis has not been made out in living cells. It is possible, of course, that the phenomenon might escape notice, especially in connective tissue cells where the nuclei are sometimes not distinct and the cell outlines not well defined. But even in stained preparations convincing figures indicating the formation of two cells following amitotic nuclear division were not found. Dumb-bell nuclei, as well as nuclei presenting knob-like prominences partially or completely constricted off, were seen both in tumor and connective tissue preparations (figure 8). Such a breaking up of the nucleus, however, seemed to result not in the formation of new cells but in the development of multinucleated cells of moderately large size.

We have made one observation which shows that cells with two nuclei, such as are seen not uncommonly in culture preparations, may be the result of mitotic division of the nucleus without cytoplasmic division. In the instance referred to, a cell under observation in a warm chamber exhibited all the phases of mitosis up to constriction and division of the cytoplasm. Within half an hour long pseudopods had been thrown out. At this point the preparation was fixed and stained and the fact established that two nuclei of normal size and appearance were present.

The question as to why cancer cells divide in an abnormal manner has interested those who have studied the phenomenon. The theories of von Hansemann and of Farmer, Moore, and Walker are largely concerned with the answer to this problem. The results of some experiments by Häcker (10) and Galeotti (5) are perhaps significant in this connection. Galeotti describes atypical mitoses similar to those seen in cancer cells in the epidermis of the salamander produced through the action of quinine, antipyrine, and other agents. Häcker obtained similar results by subjecting the eggs of cyclops to ether. The suggestion was made that in mouse cancer at least the atypical mitoses described might have been the result of ether narcosis. This idea was readily refuted by Bashford.

Ribbert (2), looking for a more tangible explanation, suggested that malnutrition occasioned by the poor circulation in tumors might be responsible for the abnormalities in division. It is doubtful if the present studies throw any light on the question. With Ribbert's explanation in mind it may be suggested that the conditions of food supply are the same for cultures of both kinds of tissue, in one of which abnormal mitoses are present, in the other, absent. It must be remembered, however, that abnormalities in structure, developed during the growth of cells in the body, could not be expected to disappear in a succeeding generation *in vitro,* even though the causative factor might conceivably be removed.

RATE OF DIVISION.

As indicated above, the chromatin material is clearly visible in the cell during division. The earliest changes in the nucleus are not always easily made out, but with proper lighting and practice in making observations the process can be followed through all phases.

We pointed out previously (11) that under constant conditions the time required for connective tissue cells of the rat to divide did not vary greatly. For example, at body temperature (38° C.) they require, as a rule, twenty-five to forty-five minutes. We found that the time required varied with the temperature, and that cells of different species show somewhat different time periods.

In the case of sarcoma cells, however, we have found that a marked variation in the division rate obtains, even among cells in the same preparation. The time is rarely shorter than that required by connective tissue cells, but two to four hours may be occupied in the process. Table I contains records of observations upon mitosis in ten sarcoma cells and an equal number of connective tissue cells.

TABLE I.

Rat Connective Tissue Cells.

No. of animal.	Temperature, C.	Days [illegible]	Mitosis began.	Separation of daughter chromosomes.	Beginning construction of cytoplasm.	Division completed.	Time required in minutes.
1	34°	15	9.10	9.35	9.38	9.45	35
2	34°	15	9.20	9.51	9.54	10.01	40
3	34°	78	5.39	6.10	6.12	6.20	50
4	36°	15	8.35	9.07	9.09	9.15	40
5	36°	4	8.34	9.09	9.11	9.16	42
6	38°	20	3.45	4.07	4.10	4.13	28
7	38°	40	9.30	9.45	9.47	9.52	22
8	38°	20	11.03	11.21	11.24	11.29	26
9	39°	10	2.18	2.41	2.44	2.47	29
10	39°	10	2.10	2.26	2.28	2.31	21

Rat Sarcoma Cells.

1	35°	5	10.00	11.00	11.03	11.12	72
2	35°	3	9.15	10.34	10.36	10.40	85
3	37°	3	9.20	9.42	9.45	9.55	30
4	37°	4	8.03	Complete disintegration at 10:15			
5	37°	3	3.15	4.43	4.45	4.52	97
6	38°	3	7.20	7.38	7.40	7.45	25
7	38°	2	9.45	10.44	10.47	10.55	70
8	38°	2	10.20	10.45	10.49	11.04	44
9	38°	3	10.30	Complete disintegration at 12.30			
10	38°	8	2.10	2.25	2.27	2.34	24

Occasionally a cell is seen in which mitosis appears to progress normally to the formation of the equatorial plate and then stops; the nuclear material becomes gradually condensed into a translucent mass, and, after one to three hours rapid disintegration of the cell ensues. This phenomenon is striking. The cell suddenly bursts; a number of small rounded fragments constitute the debris. The course of division in the later phases in sarcoma cells is usually the same as in connective tissue cells; that is, within two or three minutes after the separation of the masses of daughter chromosomes, constriction of the cell body begins, division of the cytoplasm being completed in five to ten minutes. Pseudopods are immediately thrown out by the daughter cells, which within a half hour have become indistinguishable from the neighboring resting cells.

CONCLUSIONS.

1. In primary cultures sarcoma cells exhibit a much greater activity than do normal connective tissue cells grown from the adult blood vessel; there is a shorter latent period, ameboid phenomena are more marked, and cell multiplication proceeds more rapidly.

2. In secondary cultures sarcoma cells are less active than in primary cultures; connective tissue cells, on the other hand, show a markedly accelerated growth.

3. Connective tissue cells are more easily propagated over long periods *in vitro* than are sarcoma cells; they multiply actively in cultures more than three months old.

4. The method of tissue cultivation is well adapted to the study of normal and pathological cell division; the nuclear changes are easily discernible in the living cell as division proceeds, and staining methods may be applied to verify observations upon the unstained structures.

5. Atypical mitoses of several kinds are found in cultures of sarcoma cells but are not seen in growths of connective tissue.

6. The time required for division in rat connective tissue cells kept at body temperature (38° C.) varies within relatively narrow limits (twenty to fifty minutes); sarcoma cells, on the contrary, exhibit marked variations and several hours may be required.

7. In studies upon living cells amitotic division has not been observed in either normal or tumor tissue. Evidences of nuclear budding, however, with the formation of cells containing several nuclei of irregular size have been noted.[3] The development of a cell with two nuclei from a mononuclear cell by mitotic division of the nucleus without division of the cytoplasm has also been observed.

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EXPLANATION OF PLATES.

PLATE 68

FIG. 1. A field in a three day culture of mouse sarcoma. In the upper part of the picture cells are seen wandering along the cover-glass in an area from which fibrin has retracted. In the lower part cells are seen moving through the fibrin meshwork. Seven mitoses are visible; one, on the left, is a large hyperchromatic cell.

FIG. 2. An area containing a tongue-shaped group of cells in a fifteen day secondary culture of rat blood vessel. The four dividing cells represent several phases of karyokinesis: (*a*) prophase; (*b*) metaphase, with complete withdrawal of pseudopods; (*c*) telephase, showing segregation of daughter chromosomes, constriction of cytoplasm, and beginning extrusion of pseudopods; (*d*) two daughter cells immediately after division, showing compact, deeply staining nuclei.

[3] Cells of the type here referred to are not to be confused with the large multinucleated giant cells formed about foreign bodies or on the cover-glass in cultures of chick or rat spleen, the mechanism of the formation of which was discussed in a former paper (Lambert, R. A., *Jour. Exper. Med.*, 1912, xv, 510).

PLATE 99.

FIG. 3. Three mitoses from a four day preparation of rat sarcoma, showing no abnormality in structure; (*a*) a cell with the spindle peripherally situated; (*b*) normal mitosis; (*c*) a cell flattened on the cover-glass; the chromosomes are well separated.

FIG. 4. Three mitoses from a three day culture of rat sarcoma; (*a*) a large hyperchromatic cell; (*b*) mitosis with lagging chromosomes; (*c*) mitosis in a cell containing a large vacuole.

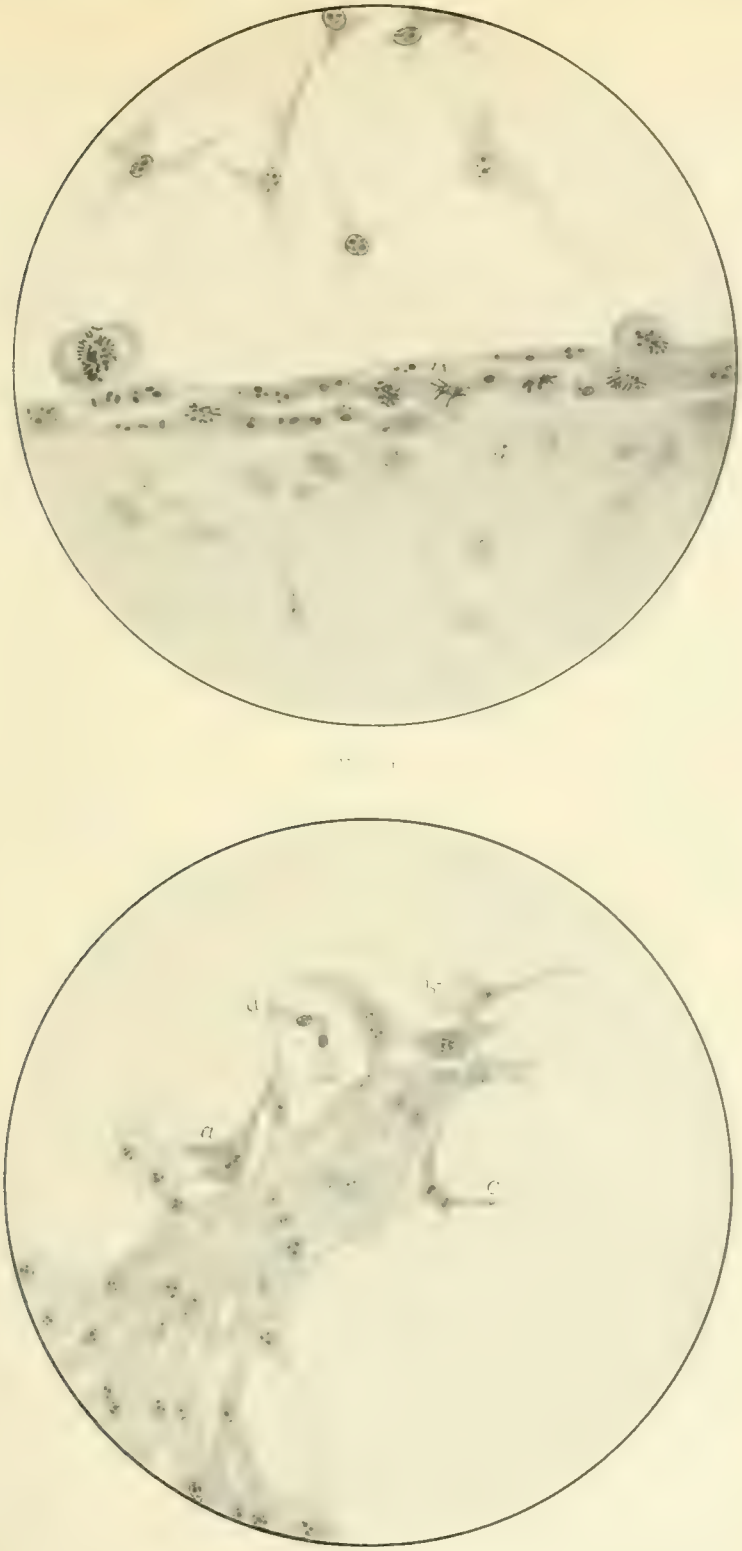
FIG. 5. Three cells from a five day growth of rat sarcoma; (*a*) bipolar mitosis with lagging chromosomes. This cell when fresh had the appearance of a tetrapolar mitosis; (*b*) tripolar mitosis; (*c*) a cell containing three newly formed nuclei, the result apparently of a tripolar mitosis without division of the cell cytoplasm.

PLATE 100.

FIG. 6. Four drawings at fifteen minute intervals of a rat connective tissue cell undergoing division, and a resting cell (*x*) for comparison. (*a*) Prophase; (*b*) metaphase; (*c*) telephase; (*d*) daughter cells fifteen minutes after division. In the living cell the chromosomes are visible in the cytoplasm on account of their slightly greater refractivity. They are represented here in dark color for the sake of clearness. The small white spaces in the cells are fat droplets.

FIG. 7. (*a*) Young connective tissue cells fixed and stained five minutes after division; (*b*) connective tissue cells fixed and stained thirty minutes after division; (*x*) resting cell for comparison.

FIG. 8. (*a*) Budding nuclei in sarcoma cells; (*b*) the same in connective tissue cells.



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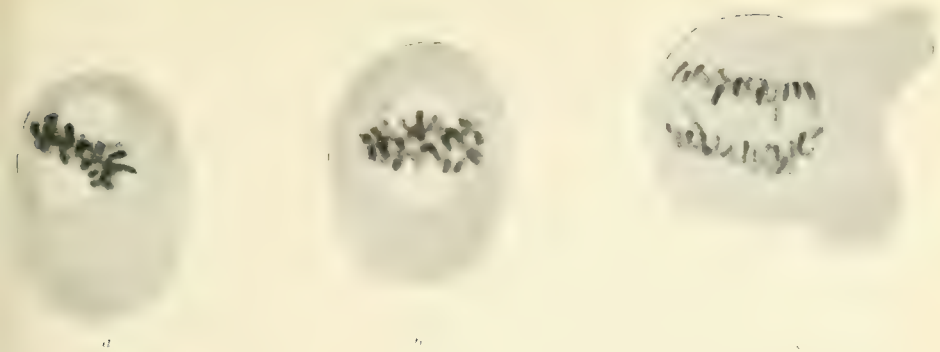


FIG. 3.

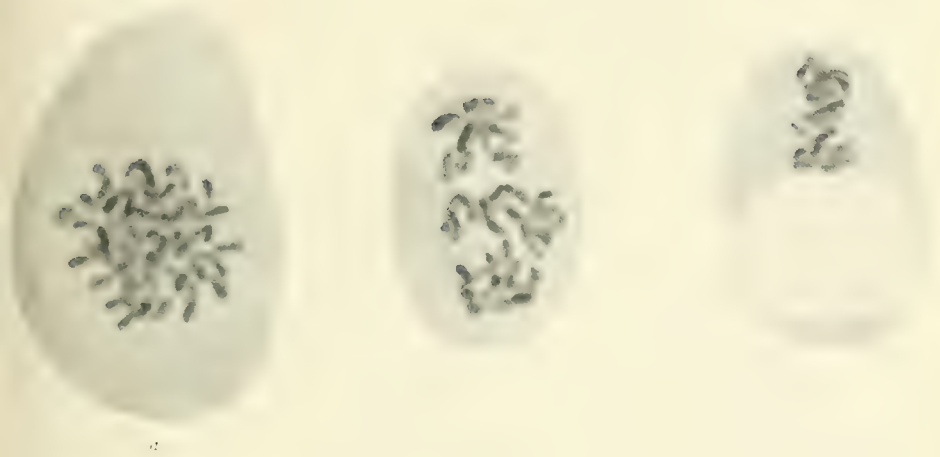
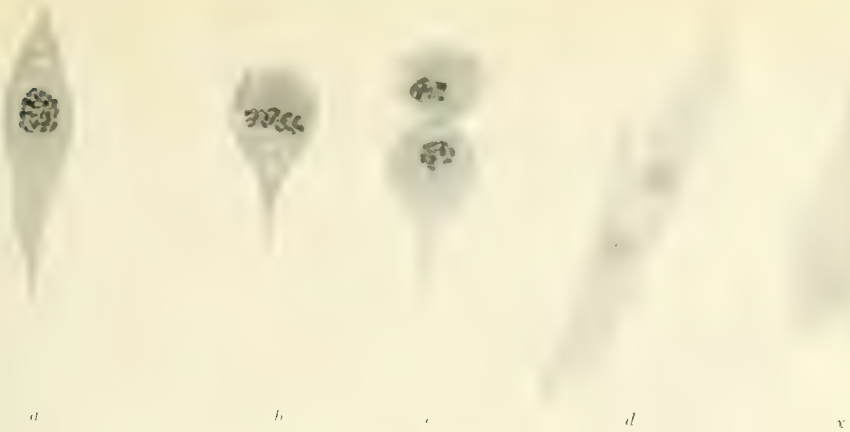


FIG. 4.

FIG. 5.

(Lambert: Cancer Cells and Normal Cells.)



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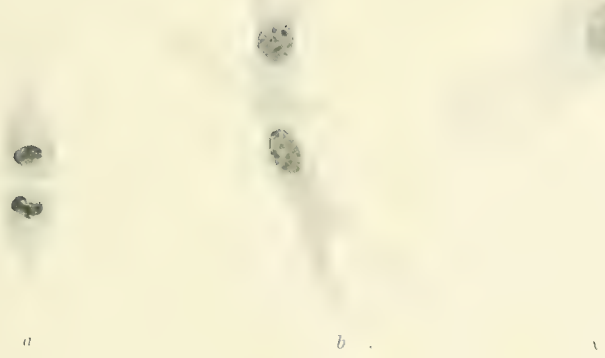

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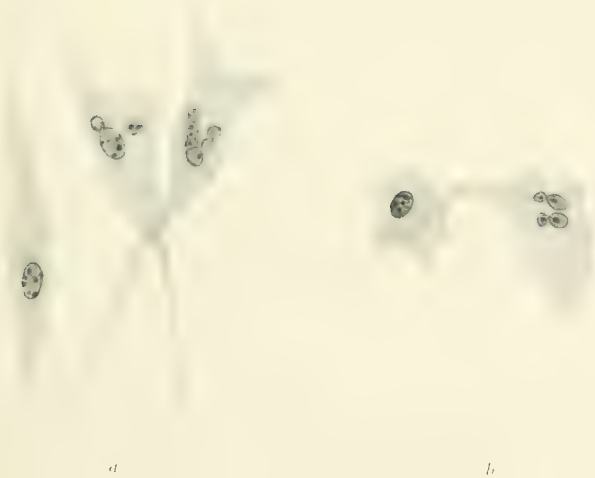

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STUDIES ON THE CULTIVATION OF THE VIRUS OF VACCINIA. II.*

EDNA STEINHARDT AND ROBERT A. LAMBERT.

From the Research Laboratory, Department of Health, and the Department of Pathology, College of Physicians and Surgeons, New York City.)

Two years ago[1] we began to apply the method of cultivating tissues *in vitro* (Harrison) to the study of the viruses of certain infections in which the specific living agents have not yet been identified. The three viruses studied thus far with the new method have been those of rabies, vaccinia, and syphilis.[2] In the study of the former we attempted, first, to determine whether the Negri bodies, the nature of which is still in dispute, would show any development or multiplication *in vitro* when fragments of brain from rabid animals were incubated in blood plasma, and, second, to see if the characteristic bodies could be produced *in vitro* by combining normal brain and virus. We found that no development or multiplication took place *in vitro*, and that, altho structures indistinguishable from certain forms of Negri bodies developed in the virus-normal brain preparations, the same bodies were found in control preparations in which were pieces of normal brain without virus. In a single instance the virus remained alive after 8 days' incubation; animal inoculations of preparations incubated for a longer time gave in all cases negative results.

During the past year we have been applying Harrison's method to the cultivation and study of the virus of vaccinia. The results of our first series of studies appeared recently in this Journal.[3] We concluded that there was a definite multiplication of the virus in tissue cultures composed of blood plasma and pieces of cornea (of rabbit or guinea-pig) to which small quantities of diluted virus were added. Successful animal inoculations were obtained from the third subculture. Histological studies of the preparations,

* Received for publication November 3, 1913.

[1] Steinhardt, Poor, and Lambert, *Jour. Infect. Dis.*, 1912, 11, p. 439.

[2] Steinhardt, *Jour. Am. Med. Assn.*, 1913, 61, p. 1810.
Steinhardt, Israeli, and Lambert, *Jour. Infect. Dis.*, 1913, 13, p. 294.

however, revealed no specific vaccine bodies. The present paper is concerned with the continuation of these experiments.

Levaditi[1] has recently applied Harrison's method with some success to the cultivation of the virus of poliomyelitis.

We wish to record at this time experiments upon the following points: (1) estimation of the extent of multiplication of the virus in cultures by use of higher dilutions of original virus; (2) necessity of *living* corneal tissue for the growth of the virus; (3) possibility of substituting other organ tissues for cornea; (4) use of plasma and cornea from immune animals in the culture preparations.

USE OF HIGHER DILUTIONS OF VIRUS.

In previous experiments, described in our first paper,[2] the virus was diluted 1:5 before use in the cultures. Inoculation of a small number of unincubated preparations in the skin of rabbits gave in some instances 50–60 pustules; the incubated preparations, on the other hand, produced such extensive confluent eruptions that the number of pustules could not be estimated with accuracy. In order to determine the extent of multiplication of the virus, it therefore seemed desirable to make use of a higher dilution of the virus. With this end in view the following experiments were carried out:

Experiment 501.—Two commercial glycerinated viruses (2190 and 2103) were dialyzed in salt solution according to the method of Poor and Steinhardt[3] and placed in icebox to allow coarse particles to sediment. Only the supernatant fluid was used.

Virus 2190 was diluted 1:15 in Ringer's solution. A large number of preparations were made with rabbit cornea and plasma and the diluted virus, using the same technic as described in our first paper. Nine unincubated preparations inoculated in the skin of a rabbit gave 10 flat pustules (sixth day). It should be mentioned that with these preparations a very dilute virus gives frequently flat pustules. This may be due to a lytic action of plasma on virus. The same number of slides, incubated 11 days, gave upon inoculation 60–65 elevated pustules. Practically all of the preparations in this series showed contamination with bacteria, and, consequently, only a slight and transient growth of corneal epithelium. Whether or not bacteria interfere with the growth of the virus in tissue cultures, we are not yet prepared to say, since contaminations have been exceptional in our experiments. In this instance the virus was probably the source of the infection.

[1] *Compt. rend. Soc. de biol.*, 1913, 74, p. 1704.

[2] *Jour. Infect. Dis.*, 1913, 13, p. 294.

[3] *Jour. Infect. Dis.*, 1913, 12, p. 202.

Virus 2193 was diluted 1:15 in Ringer's solution and preparations made as before. Nine unincubated slides inoculated in the skin of a rabbit gave 6 flat pustules. Inoculation of the same number of preparations incubated 16 days (contaminated) produced 58 well-developed pustules.

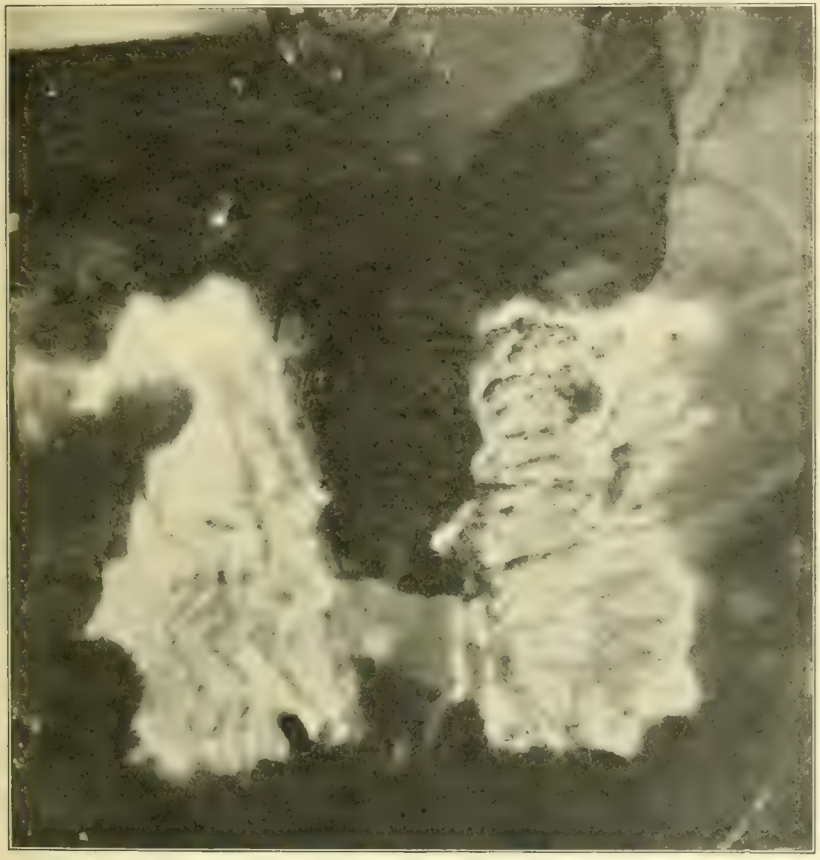

FIG. 1.—Skin of rabbit showing on its right side no "take," only scars from shaving following inoculation of five "immune" preparations; and on left side, a confluent "take" from inoculation of control preparations.

These two experiments show, then, that in spite of bacterial contamination, which may conceivably interfere with the growth of the specific microorganism, the virus was increased six- to tenfold by incubation in the cornea plasma preparations. In several other experiments in which a lower dilution of the virus was used, the extent of growth appeared to be greater, but in no

instance has the growth been comparable to that observed in cultures of rapidly growing bacteria. Irregularities in the rate of growth are to be expected, since they occur in the cultivation of practically all of the known microorganisms.

The method used here for estimating the degree of multiplication has been shown by Calmette and Guerin[1] to be reasonably accurate, comparing favorably with the method of plating for bacteria; it is in general use in commercial laboratories.

NECESSITY OF LIVING TISSUE FOR GROWTH OF VIRUS.

We have shown (first paper)[2] that when pieces of paraffin are substituted for cornea in plasma preparations, no growth of the virus takes place. To determine whether or not *living* cornea was necessary, the following experiments were carried out:

Experiment 503.—Virus 2186. Diluted 1:5 in Ringer's solution. The pieces of cornea to be used were divided into two lots; those in the first lot were frozen in an ice-salt mixture at −18° C. for 30 minutes, and were then used for virus cultures. The unfrozen tissue was used in control preparations. The frozen tissue upon incubation showed no outgrowth of either epithelium or connective tissue cells. Nine of these preparations incubated 12 days gave upon inoculation only 12–13 pustules; the same number of control preparations gave a confluent "take" composed of at least 300 pustules.

Experiment 504.—Virus 2186. Diluted 1:5. Pieces of cornea were placed for 45 minutes in weak hypotonic salt solution before being used. Inoculation of 9 unincubated preparations gave 50–60 pustules. Nine slides incubated 10 days were negative upon inoculation. There was also no "take" following the inoculation of 5 slides incubated 21 days. Microscopic examination showed that there was no growth of corneal tissue in any of the preparations. A second experiment was made at the same time using cornea killed by freezing as in Experiment 503. The results were practically identical with those just recorded for cornea killed by hypotonic salt solution.

These three experiments indicate that the vaccine virus does not grow in preparations containing corneal tissue killed by freezing or by a weak hypotonic salt solution. These two methods of destroying the vitality of the tissue were used because the chemical changes which they bring about are very slight. The results obtained are in harmony with the general conception of the close association of the virus with living body cells.

[1] *Ann. de l'Inst. Pasteur*, 1905, 19, p. 317.

[2] *Op. cit.*

SUBSTITUTION OF OTHER TISSUES FOR CORNEA.

Pieces of heart, liver, and kidney were used. Since the technic employed was the same as with cornea, it seems unnecessary to describe the experiments in detail. The results were as

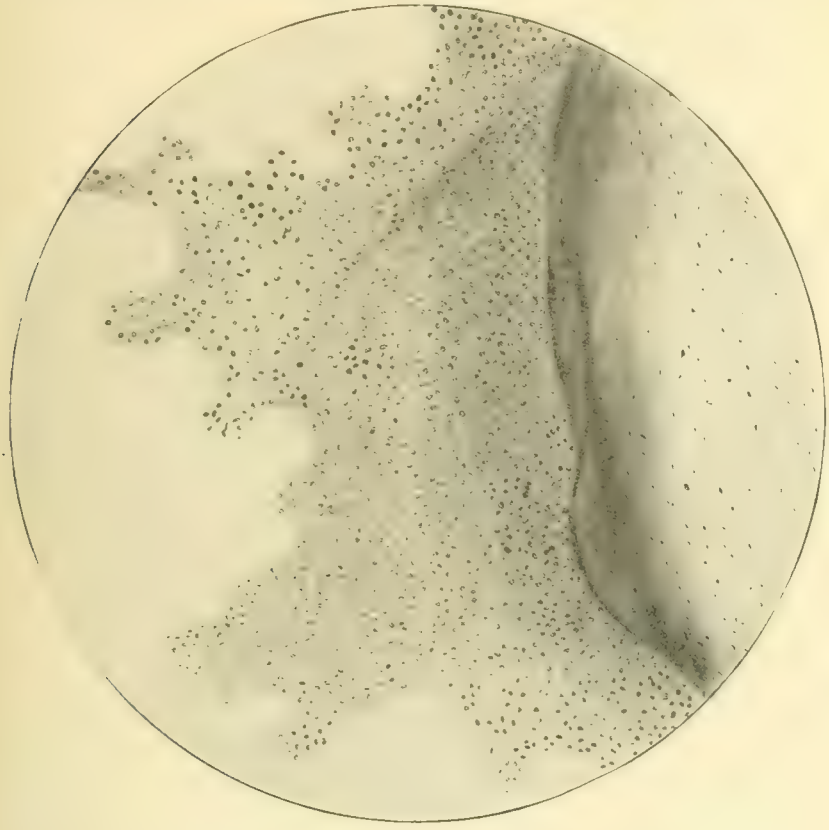

FIG. 2.—Tissue culture preparation of cornea showing outgrowth of a sheet of epithelial cells. Along the margin the sheet is only one cell thick, and the cell boundaries are quite distinct. The compact corneal fragment contains small oblong nuclei.

follows: The virus died out quickly in the liver preparations. This was due no doubt to the action of bile, which has been shown to be highly toxic for the virus. There was no evidence of multiplication in the heart and kidney preparations; on the contrary, there seemed to be a gradual weakening of the virus. Preparations

incubated for more than 10 days were found to be practically inactive.

USE OF PLASMA AND CORNEA FROM IMMUNE ANIMALS.

Immunity was established in a rabbit by a cutaneous inoculation of virus, resulting in an extensive eruption. Two weeks later the animal was bled, and the cornea removed for use in the following experiment.

Experiment 486.—Virus diluted 1:5. Two series of preparations were made: one, with plasma and cornea from an immune animal; the other, with plasma and cornea from a normal rabbit (controls). Inoculation of 5 "immune" preparations incubated 5 days gave a completely negative result (skin of rabbit, right side). Inoculation of same rabbit simultaneously on left side with an equal number of control preparations yielded a very extensive confluent "take." Repetition of this experiment a week later gave a similar result. In both experiments there were excellent growths of corneal epithelium.

This experiment shows that the virus becomes inactive in a short time in preparations containing plasma and cornea from an immune animal. The experiment also affords good proof of the adaptability of the method of tissue cultivation for the demonstration of many immunity reactions *in vitro*.

CONCLUSIONS.

1. The virus of vaccinia incubated in tissue cultures composed of plasma and cornea from normal rabbits and guinea-pigs shows a definite increase, but the degree of multiplication is not comparable to that observed in cultures of rapidly growing bacteria.

2. There is no growth of the virus in preparations containing cornea killed by freezing or by hypotonic salt solution.

3. There is no evidence of the growth of the virus in preparations in which pieces of heart, liver, or kidney have been substituted for cornea.

4. The virus is soon rendered inactive in preparations containing plasma and cornea obtained from an immune animal. The method of tissue cultivation is well adapted for the demonstration of immunity reactions *in vitro*.

THE INFLUENCE OF TEMPERATURE AND FLUID MEDIUM ON THE SURVIVAL OF EMBRYONIC TISSUES IN VITRO.

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THE INFLUENCE OF TEMPERATURE AND FLUID MEDIUM ON THE SURVIVAL OF EMBRYONIC TISSUES IN VITRO.*

BY ROBERT A. LAMBERT, M.D.

(From the Department of Pathology of Columbia University, College of Physicians and Surgeons, New York.)

The object of this report is to present briefly the results of studies upon the duration of life of embryonic tissues preserved *in vitro* at varying temperatures and in several fluid media, the method of tissue cultivation being used to determine the viability of the tissue.[1]

There are many reports in the literature concerning the survival of tissues and organs after removal from the body, especially of the tissues which are frequently transplanted surgically in human beings. Wentscher (1), for example, was able to graft pieces of human skin preserved for twenty-two days *in vitro;* Ljunggren (2) obtained positive results with skin kept for thirty days in tubes of ascitic fluid; while Carrel (3) reported recently that he had been able to transplant to other individuals pieces of skin removed from a child, after two to seven weeks' preservation in cold storage; the pieces preserved for the longer period, however, were not uniformly successful.

Similar reports have been made regarding the preservation of other tissues. Tuffier (4) transplanted omentum kept for one month in cold storage; Magitot (5) grafted corneas which he had preserved for eight days; and Davis (6) obtained good results with fascia transplanted after being stored for forty days at 32° C.

In all these instances the survival of the organs or tissues was indicated by the success of the transplantation. A clinically successful transplantation does not, however, prove that the transplanted cells were living, for Levin and Larkin (7) and Carrel (8)

* Received for publication, June 5, 1913.

[1] For details of the technique of the method, see Lambert, R. A., and Hanes, F. M., *Jour. Exper. Med.*, 1911, xiii, 495; xiv, 129.

have shown that segments of killed blood vessel may be successfully grafted, the dead tissue affording a temporary but competent tube which is gradually replaced by the tissues of the host. It may nevertheless be assumed that many adult tissues, especially skin and structures of a purely connective tissue type, are able to survive, under suitable conditions, for days or even weeks after removal from the body. But this may not be true of embryonic tissues. Indeed, Askanazy (9) found in his transplantation experiments that the tissues of rat embryos kept in the ice box for longer than eight days gave regularly negative results.

In our investigations, in which the tissues of chick and rat embryos have been used, the results as regards the possible duration of life *in vitro* and the influence of certain factors (temperature and fluid medium) on this period of survival have been so definite that it seems probable that the points brought out may have a more general application. It is rather on this account than because of the interest of the results themselves that this report is made.

EFFECT OF TEMPERATURE.

The technique used in these studies was quite simple. Small pieces of the tissue (chick or rat embryo) were preserved generally in hanging drop plasma preparations, which were carefully sealed with vaselin. The slides were divided into groups and stored at different temperatures. After varying periods of time the slides were removed, the pieces of tissue were transferred to fresh drops of plasma, and the new preparations were incubated at 37° C. This method of preserving the tissue in hanging drop slides is especially convenient and reduces to a minimum the opportunity for infecting the tissue in handling.

Two refrigerator ice boxes were used in the majority of the experiments. The temperature in them varied somewhat in different seasons, but for periods of one to three weeks the variations were not, as a rule, greater than 2° to 3° C.

The first series of experiments was carried out in midwinter when the temperature of one of the ice boxes was as low as —6° to —7° C. A series of slides containing pieces of chick embryo was

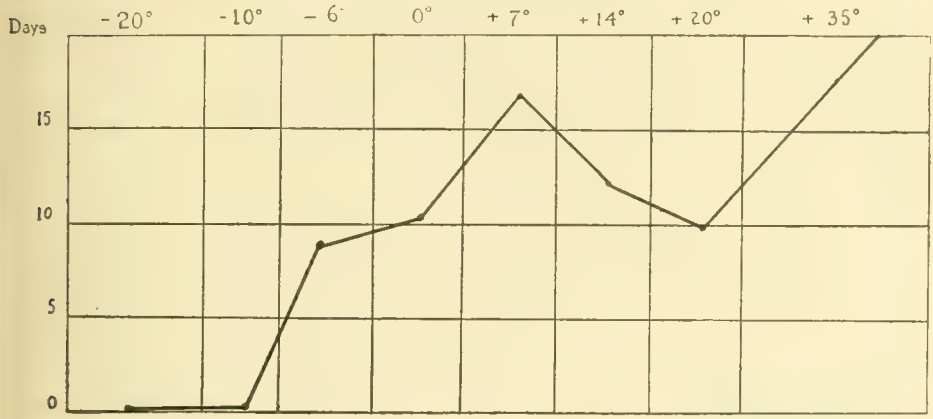
stored at this temperature; another series was placed in a north room without a radiator, in which the temperature during the course of the experiment ranged between + 12° and + 14° C. After periods of from two to sixteen days, pieces of tissue from both series were transferred to fresh drops of plasma and incubated at 37.5° C. Since the freezing point of chick tissue is about — 7° C., some of the ice box specimens were found to be frozen. The tissue from none of these grew upon incubation. Tissue from the unfrozen preparations preserved as long as six days at this temperature exhibited active growth upon incubation. Eight day preparations gave only occasional growths, while tissue preserved ten days or longer at this temperature gave uniformly negative results. Tissue kept eight and ten days at room temperature, however, showed active growth, as did also, though less regularly, twelve day specimens; sixteen day preparations were uniformly negative. Repetitions of this experiment gave practically the same results.

It was concluded, therefore, that chick tissues may be preserved longer at cool room temperature than at a temperature just above the freezing point of the tissues, and, further that the limit of survival even at the more favorable temperature is relatively short (twelve to fourteen days).

A second series of experiments was carried out later when the temperature in the two refrigerator ice boxes was respectively — 1° to 0° C. and + 6° to + 7° C. The temperature at this time in the room used varied from + 18° to + 21° C. The tissue was preserved, as before, in the form of fairly small fragments in plasma sealed in hanging drop slides. Transfers were made after varying intervals up to twenty days as in the previous experiment. The results may be briefly stated as follows: The tissue preserved for eight days at — 0° to — 1° C. showed active growth upon incubation; ten day preparations were viable although many of the transfers were negative, and the positive cultures exhibited a relatively long latent period (two to three days). Tissue preserved for a longer time at this temperature failed to grow. Tissue preserved at + 6° to + 7° C., on the other hand, was regularly found to be viable after twelve to fifteen days, and some of the sixteen and eighteen day preparations gave a fair growth upon incubation.

Tissue preserved at + 18° to + 21° C. survived for from nine to eleven days; the tissue kept for a longer time failed to grow.

Stated summarily, then, it was found that of specimens of chick tissue kept at temperatures around —6°, 0°, + 6°, + 14°, and + 20°, those at + 6° survived longest; temperatures around 0° and + 14° were about equally favorable. Since chick tissues survive freezing at —8° to —10° for a few hours at most, and exposure



TEXT-FIG. 1. Chart showing the approximate periods of survival of embryonic tissues preserved at different temperatures between —20° and + 35° C. Above 25° C. chick tissues exhibit *in vitro* active growth which may be maintained for an indefinite time. The last segment should therefore be regarded as incomplete.

to —20° for a few minutes only, and since at incubator temperatures (30° to 37°) the same tissue may be preserved in a state of active life for many weeks or even months, a curve plotted to show these variations in length of life at different temperatures would present first almost a horizontal line, then a sharp rise, then a gradual rise, then a slow decline, followed by a second rise (text-figure 1).

The experiments upon the effect of temperature were repeated with rat embryonic tissues instead of chick tissues. The findings were in general the same as with chick tissue. At 0° the period of survival was about ten days; at + 6°, a few days longer. Rat tissues were more readily killed by freezing.

EFFECT OF FLUID MEDIUM.

It occurred to us that in regard to the medium used there might be at least two factors influencing the duration of life of the tissue: first the kind of medium, and secondly the quantity of fluid used in proportion to the amount of tissue. Four isotonic fluids were used as media: plasma, unmodified or diluted blood serum, Ringer solution, and physiological salt solution (0.85 per cent. sodium chloride). The same technique was used as in the temperature experiments, except that in some instances instead of making hanging drop preparations, the tissue and fluid were placed in the bottom of the cavity slides and sealed as before. The specimens were kept at 0° and + 6° C.

It was found, in brief, that the duration of life was not appreciably influenced by the use of the different fluids. It seemed conceivable, however, that although the kind of isotonic medium might be without effect, the relative quantity of fluid used and the size of the tissue fragments might exert an important influence. It seemed logical to suppose that a larger quantity would allow the dilution of the waste products of tissue metabolism, thus favoring a longer period of survival. To test this hypothesis small pieces of embryo were preserved in dishes containing 50 to 100 cubic centimeters of Ringer solution or diluted blood serum, and entire embryos were placed in another dish containing only enough fluid to cover the surface of the embryo. Pieces of tissue were also placed in dishes wrapped in gauze moistened with Ringer solution.

Subsequent transfers of the tissue showed little or no difference in the duration of life under the several conditions. In one experiment the tissue preserved in the form of entire embryos with very little fluid survived three days longer than the tissue divided into small fragments in a large dish of fluid, a result contrary to what our working hypothesis led us to expect. It seemed probable that the better preservation in the first case was not due to the smaller amount of fluid used but to the slighter mechanical injury which the larger piece of tissue suffered in handling.

CONCLUSIONS.

1. Embryonic chick and rat tissues preserved at temperatures ranging from $-7°$ to $+20°$ C. live longest at about $+6°$ C. The duration of life under the most favorable conditions is less than twenty days.

2. The kind of isotonic medium used,—plasma, serum, Ringer solution, or normal salt solution,—does not appreciably influence the period of survival. The quantity of medium in proportion to tissue is similarly without marked effect.

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4. Tuffier, cited by Carrel, A., *Jour. Am. Med. Assn., loc. cit.*
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THE EFFECT OF DILUTION OF PLASMA MEDIUM ON THE GROWTH AND FAT ACCUMULATION OF CELLS IN TISSUE CULTURES.*

By ROBERT A. LAMBERT, M.D.

(*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*)

Plates 43 to 45.

Since the method of cultivating tissues *in vitro* will no doubt be used in investigating the action of various substances on the growth and function of cells, it is important that the influence on cells, growing under these conditions, of certain easily modified physical and mechanical factors should be understood. The importance of these factors is indicated by the fact that so simple a matter as the thickness of the hanging drop of plasma influences markedly the extent of the outgrowth of cells from the original fragment of tissue.

In a former paper[1] we discussed the influence of temperature, including the effect of variations in the temperature of incubation as well as the effects of exposure of the cultures to high and low temperatures before incubation. In the present paper we shall consider the influence of another physical factor, namely dilution of the plasma medium, on the behavior of cells in cultures. Carrel and Burrows[2] (1911) were the first to describe the effect of dilution with distilled water on cell wandering, and the subject was also discussed in a recent paper by Burrows[3] (1913). Some of the work reported in this paper was begun soon after the appearance of the experiments of Carrel and Burrows, with the object of testing the correctness of the interpretation of their results regarding the

* Received for publication, January 24, 1914.

[1] Lambert, R. A., *Anat. Rec.*, 1912, vi, 91.

[2] Carrel, A., and Burrows, M. T., *Jour. Exper. Med.*, 1911, xiii, 562.

[3] Burrows, M. T., *Tr. Cong. Am. Phys. and Surg.*, 1913, ix, 77.

stimulating action of hypotonic plasma. Later, the use of the method of dilution for the study of the accumulation of fat by cells *in vitro* was suggested to us, and further experiments with this aim in view were carried out. We shall report here the results of two sets of experiments, (1) upon the effect of dilution on the migration and multiplication of cells in cultures, and (2) upon the effect of dilution on the accumulation of fat by cells in cultures.

THE EFFECT OF DILUTION OF PLASMA ON THE MIGRATION AND MULTIPLICATION OF CELLS.

Carrel and Burrows[4] reported that the addition of a limited quantity of water to chick plasma caused a more extensive outwandering of cells in cultures of chick embryo spleen. They also observed that the addition of hypertonic salt solutions was harmful. The stimulating effect in the first instance was attributed to the hypotonic state of the medium. No experiments with isotonic solutions as diluents were made. Burrows has recently published the results of studies upon the effect of the addition of such solutions. A summary of his work is given below in connection with the results that we have obtained.

We have repeated the experiments of Carrel and Burrows with hypotonic plasma, preparing at the same time cultures in plasma diluted with serum and with Ringer's solution. In addition to chick embryo spleen, we have used for cultivation chick embryo heart, bone marrow and intestine, rat embryo skin, and mouse carcinoma.

The Effect of Dilution on the Migration of Cells.—The first experiments consisted in the preparation of cultures of chick embryo spleen in the following media: (1) undiluted plasma, (2) plasma diluted with distilled water in the proportion of 3 to 2 (the optimum medium of Carrel and Burrows), (3) plasma similarly diluted with Ringer's solution, and (4) with serum, and (5) plasma diluted with serum in the proportion of 1 to 2.

Care was taken to have the pieces of tissue of uniform size, and the drops of plasma of as nearly uniform thickness as possible. Ten preparations were made with each medium. At the end of forty-eight hours' incubation, after which time experience had

[4] Carrel, A., and Burrows, M. T., *loc. cit.*

shown that little further migration of spleen cells took place, the cover-glasses with the clots attached were placed in formalin and later mounted on slides. With a projection drawing apparatus rough drawings were made in which the pieces of tissue and the halos of migrated cells were outlined. By taking the average of the radii of the tissue fragments and the average width of the cell halos, a composite drawing for each series of preparations was constructed. While the cultures in a single series differed little from one another, the composite drawings of the several series showed certain differences (figures 1, 2, 3, 4, and 5). The most extensive migration is seen to have taken place in the plasma diluted with two volumes of serum. There is little difference in the extent of migration about the pieces of tissue in plasma diluted with the smaller quantities of serum, Ringer's solution, and water, although in each the outwandering was more extensive than in the controls in undiluted plasma. In other words, the most active migration took place in the plasma diluted with the largest quantity of fluid, and not in the medium made hypotonic by the addition of distilled water. This result is in agreement with the recent work of Burrows in which he found that the extent of migration of spleen cells in plasma diluted with varying quantities of serum increases with the amount of serum added. The effect is attributed to the diminution in the amount of fibrin in the clots. Since this reduction in fibrin results, in the beginning at least, in a coarser meshwork in the clot, the explanation of the increased migration of cells would seem to lie in the diminished resistance to the progressive movements of the cells. In order to test this explanation several other tissues were used, including chick embryo heart, intestine and bone marrow, rat embryo skin, and mouse carcinoma, the cells of which vary considerably in their migratory ability.

Briefly stated, it was found that cells which are relatively small and actively motile, such as those of the bone marrow and spleen, show increased migration in diluted plasma, whereas cells whose power of locomotion is limited, such as those of intestinal epithelium, rat embryo skin, and mouse carcinoma, which tend to spread out slowly in cultures in groups or large sheets, are not influenced by dilution of the medium. Cells which occupy a midway position

in the matter of motility, such as connective tissue cells, are only slightly influenced in this way. The mechanical explanation of diminished resistance to active cell locomotion offered by the coarser fibrin meshwork seems to us to account for these results.

The Effect of Dilution on Cell Multiplication.—An analysis of the results suggests other criteria for determining a true stimulating effect on tissue cultures than the extent of the outwandering of cells through the clot. It occurred to us that the extent of cell multiplication, as determined by the number of cells that undergo division in the preparations in a given time, would afford a much more satisfactory test. As we have pointed out, all stages of mitotic division can be observed with comparative ease in the living cell. Since division at body temperature is completed, as a rule, in from twenty to thirty minutes, the number of mitoses in a preparation may be hourly recorded without danger of counting the same cell twice. By making observations on small groups of preparations from the several series over a period of twenty-four hours or longer it is possible to arrive at a definite conclusion as to which series shows the most active multiplication of cells. We have studied cultures of chick embryo heart in pure plasma and in plasma diluted with water and with Ringer's solution in the proportions given above. There were five preparations in each set. Each preparation was examined six times during a period of forty-eight hours. The total number of mitoses for each of the three sets in the order stated was, 88, 96, and 81. In another experiment in which four observations were made the total numbers were, 76, 62, and 70. We concluded from these two experiments that dilution of plasma within these limits is without appreciable effect on cell multiplication.

THE EFFECT OF DILUTION ON THE ACCUMULATION OF FAT BY THE CELLS.

In previous communications we have referred to the appearance in the cells of tissue cultures of small droplets of fat which tend to increase in size and number with the age of the culture. After five to seven days the cells may be actually distended with the droplets.

We[5] reported observations which seemed to show that the accumulation was in all probability the result of a disturbance in cell metabolism. There was evidence that the vitality of the cells was not seriously impaired, since they were seen to move about actively and to undergo normal mitotic division. We also observed that cells growing in a thin drop of plasma accumulated less fat than those in a thick coagulum. This phenomenon can be easily demonstrated in a single preparation by shaking the drop of plasma before coagulation takes place, causing the drop to touch the slide cavity, and then by manipulating the tissue fragment so that it lies along the inner edge of the drop. The cells that grow in one direction into the thick clot accumulate a large quantity of fat in their cytoplasm, while those growing in the other direction in the thin film of medium on the cover-glass accumulate very little fat. It is difficult to say whether this difference in fat accumulation is due to the difference in the quantity of fat at the disposal of the cells, or to a difference in oxygen supply to the cells under the two conditions, as has been suggested by Burrows.

Several methods suggested themselves for testing the effect of a reduction in the quantity of fat in the medium on the amount of fat accumulated by the cells: (1) the removal of the fat by extraction with ether or chloroform; (2) the use of an artificial fat-free medium, such as agar or salt solutions, in which Lewis[6] states that embryonic chick cells will grow for a time; and (3) the use of plasma in which the amount of fat has been reduced by dilution with Ringer's or physiological salt solution. The first method was found impracticable because prolonged shaking of serum with pure ether or chloroform seemed to produce some change, probably in the serum proteins, as the result of which the serum even when combined with sufficient plasma to cause clotting did not afford a satisfactory culture medium. The second method could not be successfully used, because, after washing the tissue fragments in salt solution to remove the tissue lymph before preparing the cultures, it was found that the cells in the outgrowths lived only eighteen to twenty-

[5] Lambert, R. A., and Hanes, F. M., *Virchows Arch. f. path. Anat.*, 1913, ccxi, 100.

[6] Lewis, M. R., and Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 126.

four hours; that is, not long enough to make comparative observations. Very little fat is present in the pure plasma preparations at the end of twenty-four hours' incubation. We therefore resorted to the use of the third, or dilution method. Dilutions of chick plasma in Ringer's solution were made in the following proportions: 1 part of plasma to 2, 5, 10, 12, 15, and 20 parts of Ringer's solution. Clotting was not interfered with by the high dilution of the plasma, although the coagula formed by drops of the 1 to 15 and 1 to 20 dilutions were often not firm.

It was found that cells from pieces of chick embryo heart, previously washed in salt solution, showed active migration and multiplication in each of the diluted media. However, those growing in the 1 to 20 medium underwent early disintegration, frequently in less than twenty hours. The lower dilutions only were therefore used in the majority of the experiments. A single protocol will be sufficient to show the effect of dilution on the accumulation of fat by the cells; for it was found that when proper care was exercised in the preparation of the cultures, including the thorough washing of the pieces of tissue to prevent a relatively great modification of the dilution from the addition of tissue lymph, and in the care of the instruments and glassware used, the results in a number of experiments were practically uniform.

Experiment iii–5.—Plasma was obtained by bleeding a fowl from the wing vein. The heart of a ten day chick embryo was cut into small pieces of the size suitable for culture preparations. Dilutions of plasma were made by adding 1 part to 2, 5, 10, 12, and 15 parts of Ringer's solution. Ten pieces of tissue were put up in each medium, including undiluted plasma.

All preparations upon examination after twenty-four hours showed the usual radial extension of spindle and irregularly shaped cells. Small droplets of fat were visible in the cells in pure plasma and in those in the 1:2 and 1:5 dilutions; only occasional granules were seen in the preparations of the remaining series. After forty-eight hours the differences were more striking. There was a marked increase in the number and size of the granules in the cells in pure plasma and in the 1:2 dilution. In the 1:5 dilution the cells showed a moderate amount of fat, but decidedly less than those in undiluted plasma. Those growing in the higher dilutions were practically free from fat. Three of the preparations from each series were fixed in formalin at this time in order that the observations on the living cells could be verified by staining with Sudan III (figures 6 and 7). Some of the preparations in diluted media became disintegrated on the third and fourth days. Those remaining alive as long as four days presented striking pictures when compared with the controls, the former

showing only occasional granules of fat, while in the latter the cells appeared distended with numerous large droplets.

In order to exclude the possibility that in the diluted plasma the diminution in the amount of fibrin might have influenced in some way the fat metabolism of the cells, plasma diluted with serum instead of salt solution was used in another set of experiments. It was found that the accumulation of fat under these circumstances was practically the same as in undiluted plasma. Repetitions of these experiments, including several in which pigeon spleen and mouse carcinoma were used, gave results that varied little from those just stated. Two further observations, however, should be mentioned. First, it was occasionally observed that some of the cells that wandered farthest into the clot of highly diluted plasma showed a small number of rather large fat droplets, while the cells in the denser outgrowth immediately about the tissue fragment were practically fat-free; secondly, in a few of the preparations in diluted plasma in which the outgrowth consisted of only fifteen to twenty cells, these cells contained more fat than those in preparations showing a luxuriant outgrowth. These observations seem to be in harmony with the findings stated, for it may be assumed that if the number of cells in a culture or in a particular area of the clot is small the fat supply for these cells is relatively greater.

It has therefore been concluded that the effect of dilution of the plasma with salt solutions reduces the quantity of fat accumulated by the cells by reducing the quantity of fat in the medium.

SUMMARY.

1. Dilution of plasma with isotonic solutions causes a more extensive migration in cultures of cells of the actively migratory type, such as those of spleen and bone marrow. Dilution with a limited quantity of distilled water produces the same effect. Less actively motile cells are influenced little or not at all by dilution. The effect on cells of the first type is probably due to the reduction in the quantity of fibrin in the clot producing lessened resistance to cell locomotion.

2. Dilution of plasma with either isotonic solutions or distilled water is without effect on cell multiplication, as is shown by records of the number of mitoses in living culture preparations.

3. Dilution of plasma with suitable quantities of Ringer's solution causes a marked diminution in the quantity of fat accumulated by the cells. This reduction is to be attributed to the decrease in the quantity of fat in the medium. The accumulation of fat by cells in cultures is therefore not to be regarded as the result of a cell degeneration, but as an accumulation, the source of the fat being the medium in which the cells are growing.

EXPLANATION OF PLATES.

Plate 43.

Drawings showing the extent of migration of spleen cells in undiluted chick plasma and in plasma diluted with serum and with salt solutions.

Fig. 1. In undiluted chick plasma (control).

Fig. 2. In plasma diluted with serum in the proportion of 3 : 2.

Fig. 3. In plasma diluted with distilled water in the same proportion.

Fig. 4. In plasma diluted with Ringer's solution in the same proportion.

Fig. 5. In plasma diluted with serum in the proportion of 1 : 2.

Plate 44.

Fig. 6. Three day culture of chick embryo heart in undiluted plasma. Stained with hematoxylin and Sudan III to show the accumulation of fat by the cells.

Plate 45.

Fig. 7. Three day culture of chick embryo heart in plasma diluted with Ringer's solution in the proportion of 1 : 15. The staining shows that fat granules are practically absent. It is also to be observed that the cells are smaller and stain somewhat more deeply with hematoxylin than in the control preparation.

FIG. 1.

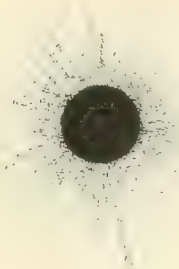

FIG. 2.

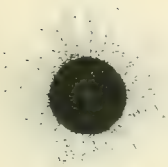

FIG. 3.

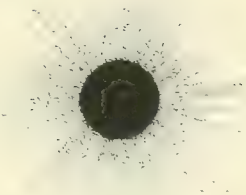

FIG. 4.

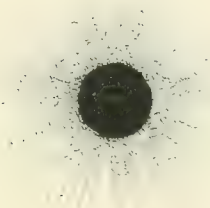

FIG. 5.

(Lambert: Growth of Cells in Tissue Cultures.)

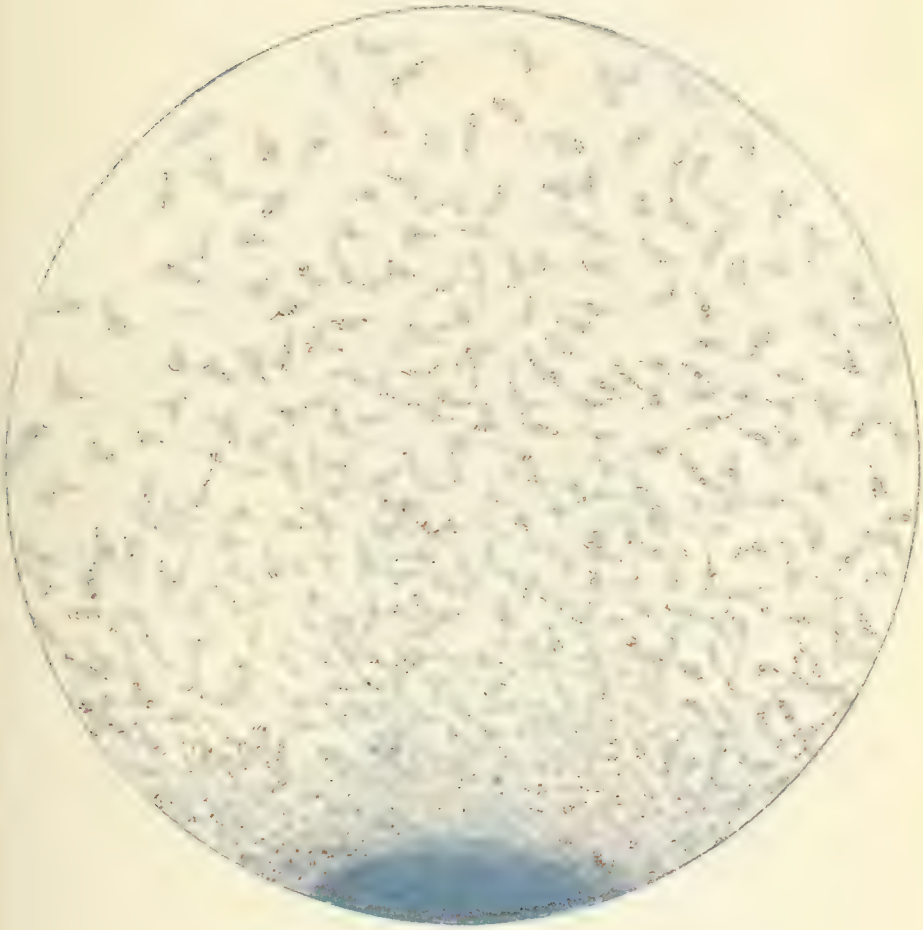

FIG. 6.

(Lambert: Growth of Cells in Tissue Cultures.)

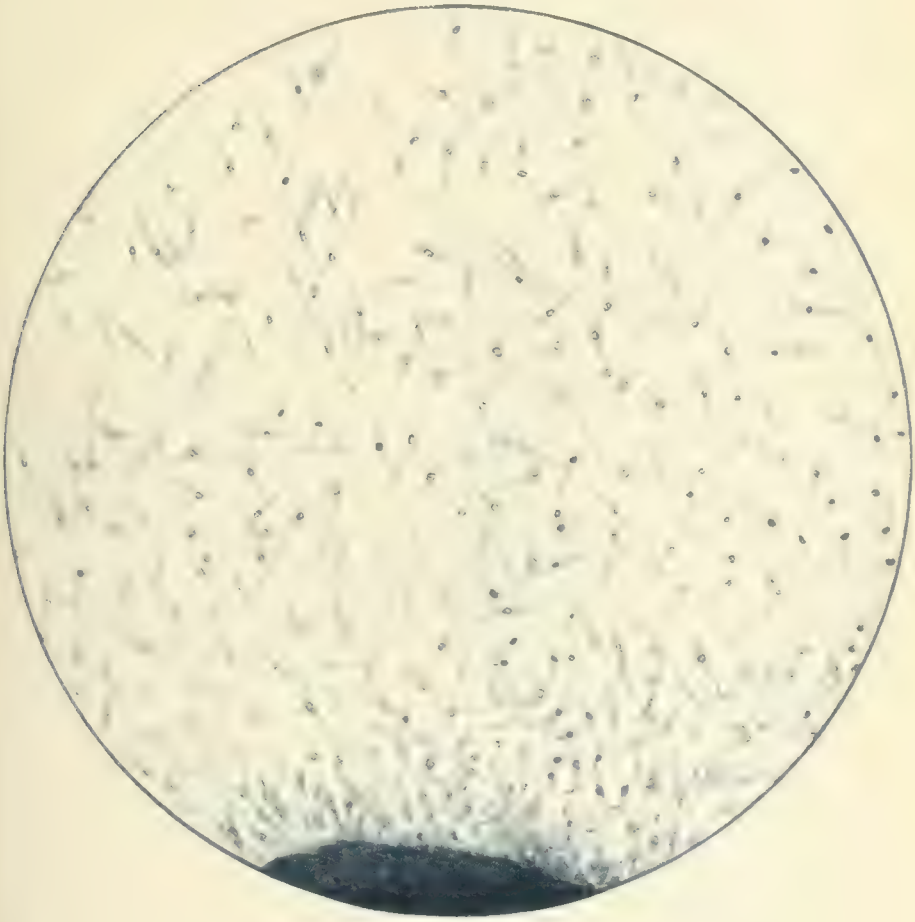

FIG. 7.

(Lambert: Growth of Cells in Tissue Cultures.)

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A NOTE ON THE SPECIFICITY OF CYTOTOXINS.*

By ROBERT A. LAMBERT, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

Since Metchnikoff and Landsteiner, working independently, demonstrated in the serum of rabbits treated with guinea pig spermatozoa the development of substances which immobilized and dissolved spermatozoa more or less completely, many attempts have been made to obtain cytotoxic sera specific for the different types of body cells.

It was early shown by von Dungern (1) that if rabbits were injected with the tracheal epithelium of cattle, the serum of the treated animals developed the power of causing the rapid disintegration of epithelial cells of that kind. He showed further that the serum was lytic for bovine erythrocytes, but that the antibodies possessed a greater affinity for tracheal epithelium. Metchnikoff (2) found that his spermatoxic serum was likewise hemolytic, but he maintained that the hemolytic power developed as the result of the injection of hemolytic receptors. Later investigators have obtained cytotoxic sera by injecting into animals of a foreign species the cells of practically every tissue of the body; leucocytes (Metchnikoff (3), Besredka (4)), liver (Delezenne (5), Deutsch (6)), kidney (Lindemann(7), Néfédieff (8), Bierry (9), Ascoli and Figari (10)), pancreas (Surmont (11)), adrenal (Bigart and Bernard (12)), thyroid (Gontscharukov (13), Mankovski (14)), heart muscle (Centanni (15)), ovary (Ceconi and Robecchi (16)), nervous tissue (Delezenne (17)), (Centanni (18)), syncytium (Ascoli (19), Liepmann (20)), etc.

Several methods have been employed for testing the immune sera as regards their specificity for the particular cells used as antigens. The methods of exposing cell emulsions to the action of the serum, as used by von Dungern in his trichotoxin studies, and by Flexner and Noguchi (21) in studies upon the action of snake venom on various organ cells, has not been found satisfactory for testing the specificity of cytotoxins. This may be due to the fact that one is dealing with the action of antibodies on dead and dying cells instead of on living functioning ones.

The technique which has been generally used consists in injecting the immune serum into the animal body, either subcutaneously, intraperitoneally, intravenously, or into arteries leading to particular organs. Functional disturbances and histological changes in various organs have served as criteria for determining the degree of specificity. More recently several other methods have been used, especially complement deviation and the epiphanin reaction. The results with the use of these methods are briefly summarized below.

Cytotoxic sera obtained by injecting the cells of an organ into an animal of a

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foreign species are relatively specific for the species from which the antigen was derived; that is, serious or fatal symptoms develop when animals of that species are treated with suitable quantities of the serum. Animals of other species are not, as a rule, so affected. These antibodies are spoken of as showing species specificity. Reports are conflicting as to whether or not they are to any degree specific for the particular organs or tissues used as antigens. That an absolute organ specificity does not exist is generally admitted, but many authors maintain, especially from a study of the lesions found in injected animals, that a relative specificity can be demonstrated. Lüdke and Schüller (22), for example, in a recent paper describe the production in dogs of a true nephritis with insignificant lesions in organs other than the kidney by the injection of the serum of rabbits immunized with dog kidney. Pearce (23), however, called attention to the fact, several years ago, that often the most striking lesions that one finds after the injection of a cytotoxic serum may be referable not to a direct toxic action on certain parenchymatous cells, but to the hemagglutinative and hemolytic properties of the serum, causing thrombosis, embolism, and hemorrhages, with secondary necroses in organs. Pearce argues further that since the cells of different organs evidently have certain receptor characteristics in common, it is hardly conceivable that specific somatogenic cytotoxins can be produced.

Beebe (24) has suggested that by the use of nucleoproteids instead of whole cells as antigens a closer approach to specificity can be reached. Wells (25), however, has recently pointed out that Beebe's suggestion is based on the false assumption that the nucleoproteids constitute the most important and most specific part of the cell.

Apart from its biological interest the problem is important on account of its bearing on the possibility of treating certain diseases, particularly cancer and exophthalmic goitre, with specific cytotoxic sera.

EXPERIMENTAL PART.

We have used a technique which appears to possess some advantages over previous methods; namely, the cultivation of tissues *in vitro*. By this method, as was suggested in a former communication (26), active living cells may be exposed to the action of immune sera, under conditions where the effects of such disturbing factors as agglutination, thrombosis, and hemorrhages can be eliminated.

Unfortunately the tissues that have been generally used by workers with other methods—liver, kidney, and other parenchymatous organs,—can not be cultivated satisfactorily in tissue cultures and therefore can not be utilized in experiments with the new technique. After a consideration of the tissues that might be used, rat sarcoma and rat embryo skin were chosen.

The next consideration was the choice of a foreign species which could be used for immunization against these tissues. It was necessary that two conditions be fulfilled: first, the plasma from normal animals of the species should be a suitable culture medium for rat tissues; and secondly, the animals should react well to the injections, that is, they should develop a strong cytotoxic serum. Guinea pigs fulfilled these two conditions. Two groups of guinea pigs were therefore injected, one with sarcoma, the other with rat embryo skin. Plasma from the two sets of animals was later used for tissue cultures for both sarcoma and skin.

Experiment 1.—The tissue for injection was prepared as follows: A sarcoma rat was bled to death and the tumor removed aseptically. The tumor tissue, which was extremely friable but not necrotic was cut up with scissors and washed with physiological salt solution to remove the excess of blood. The embryo skin was obtained from embryos near term. The skin was carefully dissected off, washed in salt solution, and finely divided. Very little blood was present.

Guinea pig 1 was inoculated subcutaneously through a Bashford needle with 0.15 gm. of rat sarcoma. Guinea pig 2 received a similar amount of embryo skin. Ten days later a second injection in each animal was made. Twelve days after the second injection each animal was bled according to the technique previously described (27) and plasma was obtained for tissue cultures. A normal guinea pig was also bled to obtain plasma for control preparations. Six series of preparations were made composed of rat sarcoma and rat embryo skin in plasma from each of the three guinea pigs. Each series was composed of fifteen preparations. They were incubated at 37° C. and examined daily. The controls of both sarcoma and skin in normal guinea pig plasma showed after twenty-four hours an active outwandering of cells among which mitotic figures were frequent. At the end of forty-eight hours growth was luxuriant in practically every preparation. The preparations containing immune plasma were, on the contrary, much less active, though the majority showed, especially on the third and fourth days, a fair outwandering of cells. A slight but definite difference was noted in the behavior of both kinds of tissue in the plasma from the two treated animals. For each tissue there was a more marked inhibition of growth in the plasma from guinea pig 1, injected with sarcoma, than in the plasma from the animal treated with embryo skin.

Experiment 2.—In order to secure a higher degree of immunization than was obtained in the first experiment, it was decided to use larger doses of tissue for injection. Guinea pig 3 received subcutaneously 0.65 gm. of rat sarcoma. Guinea pig 4 received a similar quantity of embryo skin, prepared as in experiment 1. Twelve days later plasma was obtained in the usual way from each animal, and from a third control normal guinea pig. Small pieces of sarcoma and skin were put up in the three kinds of plasma, making six sets, each composed of fifteen preparations. Examinations were made at the end of twelve hours and daily up to the sixth day of incubation.

The controls of both tissues grew well as usual, active outwandering of cells being noticed at the end of twelve hours. There was marked inhibition in the preparations of all four sets containing immune plasma. Many of the pieces of sarcoma and skin in plasma from both immune animals not only showed no growth but presented an amorphous granular appearance. Some of the preparations, however, showed after two days an outwandering of a small number of cells. As in experiment 1, a definite difference in the toxic power of the two immune plasmas was observed. In this instance, however, the more toxic plasma was that obtained from guinea pig 4, immunized with embryo skin. This plasma acted more strongly on both sarcoma and skin than the plasma from guinea pig 3, as shown by the more complete disintegration of the tissue fragments and the smaller number of cells which wandered out in the preparations that were positive.

Experiment 3.—The two guinea pigs used in experiment 2 received after bleeding second injections of 0.65 gm. of sarcoma and skin respectively. Ten days later they were again bled and their plasma was used for cultures of sarcoma and skin as before, with controls in normal guinea pig plasma. All the fragments of both sarcoma and skin in the immune plasma of both series showed rapid disintegration with no outwandering of cells. The controls showed excellent growths.

Experiment 4.—A guinea pig was injected intraperitoneally with 15 c.c. of defibrinated rat blood. Twenty-one days later plasma was obtained and used for culture preparations of sarcoma and skin. All preparations in normal guinea pig plasma showed active growth. Both tissues showed a feeble growth in a majority of the preparations in immune plasma. In the remaining negative preparations the disintegration of the tissue fragments was not so marked as in experiments 2 and 3.

DISCUSSION.

From the first experiment it is seen that relatively small doses of rat sarcoma or embryo skin are sufficient to induce in guinea pigs an antibody reaction such that the plasma of the treated animals becomes a poor medium for the growth of either sarcoma or skin cells. There was no evidence of even a relatively specific action on the part of the cytotoxins formed, the plasma from each animal exerting an equal inhibitory action on each kind of tissue. The plasma from the animal immunized with sarcoma proved to be slightly more active against each tissue than that obtained from the guinea pig immunized with embryo.

In the second experiment in which larger doses of tissue were used for immunization a more marked toxic action was demonstrable. Again no evidence of specificity was seen. As in experiment 1 plasma from one of the animals was found to be more active against both tissues. In this case, however, it proved to be that

obtained from the guinea pig immunized with embryo. It seems clear then that these differences in toxicity are to be referred to individual differences in guinea pigs in their reacting power and not to differences in the antigenic properties of the two tissues.

The third experiment shows that a still stronger cytotoxic serum may be obtained by a repetition of large doses of either tissue. Here again no evidence of specificity was found.

The fourth experiment shows that blood may be used to immunize against both sarcoma and skin, although the cytotoxic power of such a serum seems to be not so great as when either of the other tissues is used.

A second parallel set of experiments was carried out with two chick tissues, heart and intestine, for the production of cytotoxic sera in guinea pigs. The tissues were obtained from sixteen to twenty day chick embryos. These two tissues were selected because they exhibit characteristic types of growth in tissue cultures. From the heart a radial growth of connective tissue cells is regularly seen, while from pieces of intestine wide sheets of epithelial cells stretch out. Chick tissues grow fairly well in normal guinea pig plasma. In plasma from immunized guinea pigs the results were practically the same as those obtained with rat tissues; that is, no evidence of specificity was found. The experiments are therefore not given in detail.

SUMMARY.

1. The plasma of guinea pigs treated by injections of rat sarcoma exhibits a toxic action in tissue culture preparations on the cells of both rat sarcoma and rat embryo skin. Similarly, the plasma of guinea pigs immunized by injections of rat embryo skin is toxic for cells of both types.

2. Injection of rat blood immunizes against both sarcoma and embryo skin, although not so strongly as injections of the two tissues.

3. Guinea pigs receiving injections of either chick embryo heart or intestine develop cytotoxic substances for both of these tissues.

4. The preceding findings tend to show that cytotoxins formed after the injection of different body tissues into a foreign species are to no extent specific for the tissues injected.

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EXPERIENCES WITH PROPHYLACTIC TYPHOID VACCINATION

Its Effect on Menstruation

ALBERT R. LAMB, M.D.
NEW YORK

EXPERIENCES WITH PROPHYLACTIC TYPHOID VACCINATION

ITS EFFECT ON MENSTRUATION *

ALBERT R. LAMB, M.D.
NEW YORK

Prophylactic typhoid vaccination has won for itself a well-deserved place among the great preventive measures in medicine. This advance has been accompanied by a considerable literature on the history of the procedure, the various methods employed and the results obtained. It is not the object of this article to review the literature. The facts are easily accessible,[1] and, in general, well known.

During the past two years the vaccine has been used prophylactically at the Presbyterian Hospital, New York, and it is the purpose of the present communication in reporting the results of this experience, to emphasize the facts which have seemed most important and to point out in some detail the especial effect of the vaccine on menstruation.

PREVIOUS CONDITIONS

Stimulated by the excellent results obtained by this procedure in the United States Army and by Richardson and Spooner in the training schools of Massachusetts, the medical board of the Presbyterian Hospital, New York, decided, in the spring of 1911, to offer this opportunity of immunization against typhoid to the nurses and attendants at the hospital. Before starting the inoculations, the hospital records were reviewed in order to ascertain the incidence of the disease among those brought into intimate contact with typhoid patients or the cultures of typhoid bacilli. Beginning with the year 1892, the date of the foundation of the training school for nurses, a period of twenty years was covered, during which

* From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

1. The following articles give the main facts of interest and contain references to most of the other important papers:

Russell, F. F.: The Military Surgeon, 1909; Boston Med. and Surg. Jour., 1911, clxiv, 1; Jour. Am. Med. Assn., 1912, lviii, 1331; 1912, lix, 1362; 1913, lx, 344; 1913, lxi, 666; Harvey Lecture, New York, 1913.

Spooner, L. H.: Boston Med. and Surg. Jour., 1910, clxii, 37; Jour. Am. Med. Assn., 1912, lix, 1359.

Davis, D. J.: Jour. Am. Med. Assn., 1912, lviii, 537.

Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1364.

fifty-two cases of typhoid fever in nurses, doctors, orderlies and bacteriologists were treated in the hospital. Only those cases were included which gave a direct history of contact with typhoid patients or cultures. This gave 2.6 as an average yearly percentage of cases in which typhoid was possibly preventable. There were four deaths, a mortality of 7.69 per cent.

The cases were divided as follows: nurses, 39; orderlies, 6; bacteriologists, 4; doctors, 3.

Of the thirty-nine nurses, nineteen, or about one-half, were in training or on duty in the hospital at the time of contracting the disease. The remaining twenty were caring for typhoid patients when taken ill. There were no deaths among the nurses who developed the disease while in the hospital, but of those who contracted the disease outside, two died. Both were graduates of the hospital. All six orderlies were employed at the hospital in wards in which the care of typhoid patients was necessitated, and there were two deaths. The four bacteriologists developed the disease while working with typhoid cultures. Only one was on duty at the hospital at the time of contracting the disease. The others came from other laboratories. There were no deaths. Of the doctors, two were interns at the hospital and one was a substitute. There were no deaths.

The occurrence of the fifty-two cases by years was as follows:

1892	1	1902	1
1893	0	1903	3
1894	0	1904	4
1895	1	1905	5
1896	2	1906	3
1897	0	1907	6
1898*	11	1908	1
1899	1	1909	0
1900	4	1910	3
1901	4	1911	2

* Spanish War.

It will be seen that in four separate years, 1893, 1894, 1897 and 1909, there were no cases, and that for one period of two years there was also a clean record, facts which must be borne in mind in estimating the value of the results obtained through the use of the vaccine over a comparatively short period of time. In 1898, the year of the Spanish War, there were eleven cases. At that time additional wards were opened for the care of typhoid patients and the hospital staff was greatly overworked. Several of the nurses treated at this time came from the army camps.

The conditions described do not differ essentially from those reported by observers in other localities. Thus Spooner,[2] at the Massachusetts General Hospital in Boston, found that there were three or four cases of

2. Spooner: Am. Jour. Pub. Hyg., 1909, xix, 616.

typhoid a year among the nurses and those especially exposed. He found, moreover, that in these patients the disease ran a course of more than usual severity with a greater number of complications and a higher mortality than the average. I did not find this true in our statistics. The mortality was less than that for the hospital in general and the cases were not unusually severe, nor did they show more complications than the average.

Davis[3] gives no definite figures for St. Luke's Hospital, Chicago, but states that "an occasional nurse has had typhoid."

Joslin and Overlander[4] investigated the conditions in six hospitals in Massachusetts for the years 1902 to 1906 and came to the conclusion that one nurse came down with typhoid for each 114 cases treated, and that "the hospital nurse in Massachusetts is about eight times as liable to contract typhoid fever as the ordinary citizen."

Hachtel and Stoner[5] give higher figures for Baltimore. They find that the nurses and attendants are from twelve to twenty times more liable to contract the disease than the ordinary citizen of Baltimore.

One does not need to cite further specific instances. While differing somewhat in the actual figures, all observers are agreed on the main fact, that nurses, doctors and hospital attendants run a considerably greater risk of contracting typhoid than the ordinary citizen, and that in spite of due precautions, a certain number of them contract the disease each year, the source of infection being the typhoid patient or articles which have come in contact with typhoid cases.

VACCINE

The vaccine used is prepared from an old culture of attenuated virulence, isolated about twelve years ago. The organisms are grown on large agar-slants (agar slanted in 250 c.c. Erlenmeyer flasks is very convenient) for twenty-four hours, washed off with sterile salt solution and heated at a temperature of 53 C. (127.4 F.) for one hour. The vaccine is standardized with the ordinary blood-counting apparatus. After sterility is assured, the vaccine is made up to the required dilution with sterile salt solution containing 0.25 per cent. phenol (carbolic acid). It is put up in small ampules, each containing enough for one dose, and in 30 c.c. vaccine bottles for use where larger numbers are to be vaccinated.

The vaccine was carefully compared with that used in the United States Army, a supply of which was very kindly sent to the hospital by Major Russell. In a large number of inoculations no difference could

3. Davis: Jour. Am. Med. Assn., 1912, lviii, 537.
4. Joslin and Overlander: Boston Med. and Sudg. Jour., 1907, clvii, 247.
5. Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1367.

be detected in the local or general reactions or in the Widal reactions. Therefore, it would appear that the hospital vaccine represents approximately the same potency and lack of toxicity as that used in the army. The vaccine should be fresh and in general should not be used after it is 3 months old, as it has been shown that deterioration begins at about this time.

DOSAGE

The usual dosage recommended is that employed in the United States Army. It consists of three inoculations at ten-day intervals, the first of 500 million, the second and third of one billion each. This method has been well tried and it has been conclusively shown that it confers a sufficient immunity for a period of at least three years. Conditions there, however, differ somewhat from those among nurses and in private practice where a reaction which would not trouble a man in the regular army is considered quite an event. The same dosage as previously noted was given in a considerable number of the cases in this series, and there were enough troublesome reactions to make one consider a variation of the dose. Of course, one's aim in giving the vaccine is to protect the patient against typhoid and not to avoid reactions; but, if one can produce the desired immunity with the smallest amount of discomfort, the cause of typhoid prophylaxis has been aided just so much. The aim, then, should be to inject the maximum number of bacilli with the minimum amount of discomfort and inconvenience to the patient.

One class of nurses received the four doses, recommended by Spooner,[6] of 100 million, 200 million, 400 million and 600 million at five-day intervals. These cases had fewer and less troublesome reactions than the others, but they received only about one-half the total number of bacilli given by the army method. By considering certain general factors to be mentioned presently, one can considerably increase this total without a corresponding increase in the reactions. It is generally quite safe to make the initial dose 200 million and then, being governed by the reactions which occur, one can very often make the second dose 300 to 400 million, the third 500 to 600 million and the fourth 800 million to one billion. In this way one can more nearly approximate the army total dosage without as many disagreeable reactions.

Some people prefer to take their chances on the possibility of having a reaction rather than submit to the inconvenience of a fourth injection, and in vaccinating large numbers, the extra inoculation means considerable added expenditure of time, especially among women in whom the avoidance of the menstrual period causes many delays and much straggling out of the inoculations. Therefore, it has been the more recent routine to use three graduated doses at from seven-day to ten-day

6. Spooner: Jour. Am. Med. Assn., 1912, lix, 1359.

intervals, the first of 300 million, the second of 700 million and the third of one billion. Here, as in the preceding case, one can be governed by the patient's general condition and can often without fear of undue disturbance, make the first dose 350 to 400 million and the second 800 million.

Of course, it is impossible to predict accurately what reaction a person will have, but one can be governed by certain general facts. In the first place, women are more apt to have reactions than men. This fact is brought out in the statistics of Hachtel and Stoner,[7] and was shown in the first twenty-three cases of this series, of whom twelve were doctors and eleven were nurses. The mild and moderate reactions were about twice as frequent among the latter as among the former. Members of families in which there is, apparently, a marked susceptibility to typhoid or in which the disease has run a severe course, are apt to show more marked reactions. In one family which I vaccinated, both of these facts were true, and of the five members, all showed varying degrees of reaction after all of the inoculations except a boy, aged 11. In one there was the most severe local reaction which I have seen, combined with a moderately severe general reaction and in another, the general reaction after the first two doses was sufficient to discourage a third inoculation. Persons who have a low resistance to any form of infection are apt to have troublesome reactions and the same is true of those who are run down or debilitated from any cause. Those suffering from any chronic disease, should, if vaccinated at all, receive the four smaller doses. Naturally, the preceding statements are only general principles of guidance. One will encounter perfectly robust individuals who come in none of the foregoing classes, who, nevertheless, show severe reactions, while, on the other hand, any of the types mentioned may show no reaction whatever. From my experience up to the present time, I believe that it is best to give to the persons of the types mentioned, the four doses at seven-day intervals, beginning with 200 million and increasing each succeeding dose as far as feasible. For all others, I prefer the three graduated doses, using the upper limits for each dose if possible. From a rather limited experience with children I have seen no reason for graduating the dosage according to body weight. As a rule, they stand the inoculations with less inconvenience than adults and I have seen no troublesome reactions following full-sized doses.

With many people, especially business men, a seven-day interval is preferable to one of ten days. The inoculations can thus be given on successive Saturdays, allowing them until Monday to recuperate from any ill effects. It is best where possible to give the injections in the

7. Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1367.

late afternoon or early evening so that the maximum reaction may take place while the patients are asleep.

PRECAUTIONS

From my experience it has been apparent that whatever the site chosen for inoculation the patient is inclined to wish that the vaccine had been injected elsewhere. It has been found more convenient and perfectly satisfactory to make the inoculation in the left upper arm, just above the insertion of the deltoid, using the right arm in left-handed people. The vaccine is injected into the subcutaneous tissue, care being taken to avoid entering a vein, as in such cases the general reaction is usually more rapid in its appearance and more severe, and the local reaction more troublesome. In cleaning the arm, iodin and alcohol have been found perfectly satisfactory. After withdrawing the needle, the site of inoculation is touched with iodin and no dressing of any kind is applied, as it has been found that collodion, bandages or gauze with adhesive strips simply add to the local annoyance. In exceptional cases, if the local reaction is moderately severe, a dressing of aluminum acetate has been used with good results.

In prophylactic work, one must remember that patients are not inclined to be particularly grateful for any reactions which the vaccine may cause and it is only human nature for them to attribute to the vaccine every ill for months following. As the value of typhoid vaccine is so great and the need for its universal adoption so striking, it is of the utmost importance to observe every precaution to prevent its falling into disrepute in any case. A careful history should be taken in each case and a sufficiently exact examination made to be sure that the patient is in sound health, for it is quite within the range of possibilities that a person might be inoculated in the early stages of some disease, and it would then be difficult to convince the patient or his friends that the disease was not directly caused by the vaccine. In the present work such incidents were encountered. One nurse developed a typical case of scarlet fever two days after the second inoculation, and for the first day or two even the doctor who saw her considered that she was suffering from a rather severe vaccine reaction with an associated rash. From the subsequent course of the case, there was no doubt as to the nature of the disease. In such an instance in private practice, it would be exceedingly difficult to convince the family that there was no association between the vaccine and the scarlet fever. In another case, a man had an attack of bronchopneumonia which began ten days after the third injection. At the onset he believed that his illness was in some way associated with the vaccine, though in this case the disease followed a very definite exposure to cold. Other cases of association of various diseases with the vaccine will be given later.

It is well recognized that the vaccine may cause a temporary change for the worse in an existing chronic condition. For example, Spooner[8] has encountered temporary and not serious exacerbations in chronic arthritis, chronic cholecystitis, subacute urethritis, furunculosis and acne. I have seen similar instances and will mention them later. Other observers have noted the same thing. While the exacerbations are not apt to be serious, their possibility should be recognized and all chronic conditions should be asked for in the history, as in each case one can then decide whether or not it is wise to give the vaccine. I do not believe that it should be given in cases in which there is a history of tuberculosis: but if, in spite of the existence of some chronic condition, it is decided to go ahead with the vaccine, the patient should be fully acquainted with the possibilities and the vaccination procedure should be followed cautiously and the four smaller doses administered.

Another question which arises is that of vaccination where there is a history of a preceding attack of typhoid fever. It has been found that the reactions in such cases are apt to be rather strikingly severe. In the only case of the kind in which I have used vaccination, that of a young man, there was a moderately severe reaction for a day and a half after the first dose, followed immediately by a severe attack of tonsillitis and in ten days by a profuse urticarial rash. As the disease itself confers a life immunity in almost all cases, and as vaccination in such cases is apt to be followed by bad reactions, I have since refused to inoculate patients who give a history of a previous attack of typhoid. At times this preceding history is not definite and in such cases one must exercise one's own judgment. Where there is a reasonable doubt, I believe one should proceed with the vaccination, using the four smaller doses rather cautiously.

In addition to the above precautions, one should explain the effects of the vaccination to patients and tell them just what they may expect in the way of local and general reactions. Patients should be warned to remain quietly at home while the reaction is present. The most severe reaction seen in the present cases seemed to have been increased by the patient's going about as usual on the day following the first inoculation, when the temperature was 101. The next day the temperature rose to 102.6, and in connection there was a splitting headache and slight epistaxis, while on the following day the temperature reached 100, with the accompaniment of nausea and rather severe diarrhea. Patients should also be warned against taking alcohol in any form until all signs of reaction have disappeared, for not infrequently alcohol has apparanetly added considerably to the severity of the reaction. In this connection, a curious fact was noted in three of these cases. Two patients on the

8. Spooner: Jour. Am. Med. Assn., 1912, lix, 1360.

night following inoculation and one on the second night after, when there was no general reaction and their arms had ceased troubling them, took cocktails just before dinner. Almost immediately afterward their arms, at the site of the inoculation, began to pain so severely that they could not remain at the table.

RESULTS

The inoculations were started in May, 1911. The course of procedure was carefully explained and eleven nurses and twelve doctors volunteered for the preliminary vaccinations. In these twenty-three cases, only two inoculations were given, the first of 400 million and the second, after an interval of ten days, of 650 million. In seventeen of these cases, microscopic Widals were done ten days after the first injections, the Widals prior to the vaccination having been negative in all cases at 1:20. In five the Widal was negative in a dilution of 1:20, in twelve it was positive and in two it was positive at 1:100. The results of the Widal test following the second inoculation may be seen in Table 1.

TABLE 1.—Results of Widal Test in Twenty-Two Cases*

Dilution	Reaction	Number	Percentage
1-20	Negative	3	13.63
1-20	Positive	19	86.36
1-100	Positive	12	54.54
1-200	Positive	6	27.27
1-400	Positive	6	27.27
1-1600	Positive	1	4.54

* Tests made ten to fourteen days after second inoculation.

After these preliminary inoculations, the work was taken up as a routine with each succeeding class in the training-school (Table 2). During the first few months the members of a new class are not on ward duty, and as some leave the school and others enter, the vaccinations are not started until the nurses have been here for several months. Then the class is called together, the vaccination and its importance explained and the vaccine offered to those who desire it. Vaccination is still entirely voluntary in the training-school.

The following table shows the percentage of each class vaccinated:

TABLE 2.—Percentage of Vaccinations of Nurses

Class of	In class	Number eligible	Vaccinated	Percentage
1911	29	26	16	61.5
1912	33	31	22	70.9
1913	38	32	27	84.3
1914	13	11	11	100.
1915	30	25	23	92.
Totals	143	125	99	79.2

This table shows that as the work became better known and appreciated, a larger number of the nurses wished the vaccination. Thus in the last two classes, 94.4 per cent. of those eligible for the vaccination have taken it.

Since May, 1911, 143 nurses have been in training. Of these, 99 were vaccinated, 26 refused; in 3 it was considered inadvisable on account of some form of chronic illness, 14 had recovered from typhoid and one had been inoculated with the vaccine before entering the school. Thus, 79.2 per cent. of the eligible nurses were vaccinated and 20.8 per cent. remained unprotected, including the three who wished the vaccine, but whose health did not warrant its use.

In addition, during the same period, May, 1911, to June, 1913, 33 doctors and medical students connected with the hospital, 7 employees in the pathologic department, where the vaccine is compulsory, and 64 outside cases have been vaccinated, making a total of 203 cases; and in this period there have been no cases of typhoid fever either in the training-school or among those vaccinated. There have been no untoward effects from the vaccine and no instances of arm infection.

LOCAL AND GENERAL REACTIONS

Local and General.—The local reactions were similar in all respects to those usually observed.

The general reactions are shown in Table 3. In classifying these reactions, the temperature is taken as the general index as in army cases, but some reactions are put down as mild or moderate where the constitutional disturbances were sufficient to warrant it even though the temperature remained low. It was noted that some of the nurses showed subnormal temperatures following the inoculations, which was often associated with such constitutional disturbances as dizziness, nausea and vomiting.

TABLE 3.—PERCENTAGE OF GENERAL REACTIONS

Reaction	First Dose 203 Cases		Second Dose 200 Cases		Third Dose 169 Cases		Fourth Dose 27 Cases	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
None.....	96	47.6	111	55.5	140	82.8	24	88.8
Mild.....	88	43.3	71	35.5	19	11.2	3	11.1
Moderate..	18	8.9	18	9	11	6.5	0	....
Severe.....	1	0.49	0	....	0	....	0	....

It will be noted that of these cases, 184, or 90.6 per cent., showed either no reaction or a mild one after the first dose; 182, or 91 per cent., after the second; 159, or 94.1 per cent., after the third, and 27, or 100 per cent., after the fourth. The symptoms complained of in the order of frequency were: malaise, headache, general aching, nausea, dizziness, anorexia, vomiting, diarrhea, epistaxis, faintness.

One hundred and ninety reported at the end of six months that they were perfectly well, three had suffered from fatigue for two or three

months, but had recovered, and two thought that their health was still impaired by the vaccine.

WIDAL REACTIONS

The results of the microscopic Widal in fifteen consecutive cases tested ten days after third inoculation, may be seen in Table 4.

TABLE 4.—Results of Widal Test in Fifteen Cases*

Dilution	Positive Reactions—No.	Positive Reactions—Per cent.
1-20	15	100
1-100	15	100
1-200	11	73.3
1-400	9	60
1-800	5	33.3
1-1600	3	20
1-2000	2	13.3
1-12000	1	6.6

* Test made ten days after third inoculation.

The Widal reaction has been so definitely worked out by so many observers that it is now used here only in exceptional cases and to control new vaccine.

MENSTRUATION

After the first few inoculations the very definite impression was gained that menstruation was apt to be affected by the vaccine. Consequently, careful records of this particular phase of the work were kept in order to determine the amount of the disturbance. A comparison of the army vaccine with that used in the hospital showed no difference in their liability to cause menstrual irregularities. Other workers have briefly noted that these irregularities occur, so that one is justified in concluding that the effects are not due to the peculiarities of any one particular vaccine. A correct judgment of the part played by typhoid vaccine in menstrual disturbances is difficult and any statistics are open to several sources of error.

In the first place, it is well known that nurses are apt to have menstrual irregularities during the first few months of training, which are due to change of environment and method of living. In this work, the vaccinations were not started until the nurses had been in the hospital for from three to six months, and these nurses did not show any more frequent or more marked changes than did the nurses who had been in training for from one to three years. Again, some women are always more or less irregular. Where there was such a history, any disturbances during the course of the inoculations, unless of striking character, were not considered, and the case was put down as unaffected. In some, temporary causes other than the vaccine may have been the causal factors. It is difficult to eliminate such causes and they introduce a certain unavoidable source of error.

In compiling the records of the one hundred cases in which menstruation was studied, only those cases are put down as affected in which the evidence of disturbance by the vaccine seemed to me quite definite, after ruling out all other apparent causes and where the patients themselves felt that the condition was distinctly unusual for them.

Perhaps one can gather the best idea of the nature of the disturbances and their close association with the vaccine by a brief description of some of the more striking cases.

REPORT OF CASES

Case 1.—Vaccination finished early in June. The two following periods were missed. In August appendix was removed and menstruation did not occur again until December. It has been normal since then.

Case 2.—For three periods following the vaccination was about three weeks late each time. Then normal.

Case 3.—Missed period following vaccination and then a condition of scanty flow a week or more late existed for three months.

Case 4.—Menstruated every two to three weeks for three months following the vaccination.

Case 5.—After the third inoculation came on two weeks ahead of time and caused more discomfort than ever before.

Case 6.—Vaccinated the day after menses had ceased. Menses returned again that night.

Case 7.—During the course of vaccination menses came on one week ahead of time, causing patient to go to bed.

Case 8.—Twelve days early. More profuse and of longer duration.

Case 9.—After first inoculation menses ten days early, more painful and scantier.

Case 10.—After third inoculation menses ten days early, more painful and scantier.

Case 11.—Never quite regular. Much worse after vaccination. Skipped two months entirely and later menstruation was more painful.

Case 12.—Five days late after the second inoculation. One week early after the third.

Case 13.—Skipped two periods. Next time in three weeks. Then in less than three weeks.

Case 14.—Skipped three periods.

Thus in fourteen cases there were very distinct changes, due, in all probability, to the effect of the vaccine itself.

A complete tabulation of all the cases shows that a little more than one-half, 53 per cent., showed some type of menstrual disturbance, distinct though at times quite trivial, while 47 per cent. were unaffected. The types of disturbance may be seen in the following table, more than one type occasionally appearing in the same individual:

Menstruation early for one or more periods	25
Menstruation more painful for one or more periods	18
Menstruation late for one or more periods	15
Menstruation more profuse for one or more periods	10
Menstruation skipped for one or more periods	7
Menstruation scantier for one or more periods	6

It should also be noted that where these irregularities occur the patients are more apt to have associated with them a more troublesome general reaction.

The number of cases which show some change is rather striking, even allowing for unavoidable errors. If one merely considers the fourteen very definite instances cited, the percentage is high enough to show that a very striking relationship exists between the vaccine and menstrual disturbances, a fact which is of interest in view of the similar disturbances seen in typhoid fever. All of the cases included above were followed for at least six months. In none was there any disturbance at the end of this time. Therefore, it would appear that the changes are of only temporary significance and that they carry with them no lasting ill effects.

In this connection, one must consider the possible consequences of vaccination during pregnancy. I have a record of only one such case. In this instance, the woman's husband was ill with typhoid and much of his care fell on her. Consequently, in spite of her pregnancy, vaccination was carried out. She did not develop typhoid, nor did she have any ill effects from the vaccine. The menstrual disturbances have impressed me sufficiently, however, to make me conservative about vaccinating pregnant women. At the present time, I would not vaccinate under such circumstances, except under some special indication, such as that shown in the preceding case.

Recognizing that menstrual disturbances do occur, one naturally turns to the question of reducing them to a minimum. I believe that, barring some special indication for haste, the vaccination of women should be so arranged that the first inoculation will come a few days after a period. The second and third injections can then be given at seven-day intervals, leaving about a week's leeway before the succeeding period. If, for any reason, ten-day intervals are necessary, it is best to postpone the third inoculation until a few days after the second period. By giving the vaccine in this way, menstrual difficulties are reduced, though not entirely eliminated.

ASSOCIATION WITH OTHER DISEASES

It has already been mentioned that numerous instances of aggravation of existing chronic conditions are on record. It is, therefore, of interest in a series of cases like the present, where most of the cases can be followed, to note the relation of the vaccine to the development of various diseases.

Diseases of the Respiratory Tract.—Thirteen patients developed symptoms of some such affection during the course of the vaccinations, or so soon afterwards that they attributed them to the vaccine. Five patients developed coryza beginning within one or two days after an

injection, and in one case the cold lasted for six weeks. There were five cases in which tonsillitis began within one or two days after an inoculation. In one case bronchitis developed during the course of the vaccination, and the disease lasted for two months. One patient, already referred to, contracted bronchopneumonia ten days after the third injection. In this case there was definite exposure to cold.

Probably many of these cases were merely coincidences, though one receives the very definite impression that in some cases, at least, the patient's resistance is sufficiently lowered to favor the chances of such infections.

Appendicitis.—This condition was noted in five cases. In only one, however, was the association sufficiently close to justify suspicion of the vaccine as an accessory cause. In this case, there were moderately severe reactions associated with nausea, vomiting and diarrhea, after all three doses. Very soon after the third dose the patient began to have pain in the region of the appendix, which in the course of a short time necessitated an operation.

Tuberculosis.—Three patients in the present series have developed pulmonary tuberculosis since receiving the vaccine. In no case was there any previous history of the disease, and in none was the relation between the vaccine and the disease sufficiently close to be suggestive.

Tachycardia.—In one case there was a history of mild attacks of tachycardia. The patient had two such attacks on the eleventh and twelfth days following the second inoculation, and a third attack within five hours after the third injection. In another case, that of a man who normally had a very slow pulse, the rate doubled within a few hours after receiving the first dose. In a third case with a history of attacks of rapid heart action and arhythmia, there was no effect from the vaccine.

Furuncles.—In one instance a patient began to suffer from furuncles about two weeks after the third injection.

Scarlet Fever.—The development of a case of scarlet fever during the course of the injections has already been mentioned.

Scabies.—One patient was suffering from a rather extensive case of scabies, when he presented himself for the vaccination. He was warned that the vaccine might cause an aggravation of the skin condition, but he desired the vaccine in spite of this possibility. Much to our surprise, without any treatment for the skin affection, it almost completely disappeared during the course of the inoculations, but reappeared a little later.

A CASE OF OCCLUSION OF THE LEFT CORONARY ARTERY BY A PEDUNCULATED VEGETATION OF THE AORTIC VALVE.

ALBERT LAMB, M.D.

While instances of coronary thrombosis are fairly common, cases of true embolism of these arteries are sufficiently rare to arouse considerable interest. The effects of the two conditions are so similar that they are generally considered together.

Experimentally, much work has been done on the question of the effect of coronary occlusion. The earlier researches, which culminated with Cohnheim's experiments in the early eighties of the past century, demonstrated that the coronaries were end arteries and that the occlusion of a large branch was sufficient to cause sudden death. Since then experimental work has tended to refute these views. It would require too much time to discuss these experiments in detail. It is sufficient to note that by the work of Jamin and Merkel, of Amenomiya and of Spaltcholz and Hirsh, to mention only a few of those who have given the subject their attention, the coronaries have been shown to have visible and functionating anastamoses and that the occlusion of one of the larger branches does not necessarily result in sudden death, or, indeed, prove fatal at all.

Clinically, the same conclusion has been reached. For example, Pagenstecher ligated the descending branch of the left coronary in an accident case. The patient survived five days. Fraenkel had much the same experience. West, Chiari, Merkel, Dock, Recklinhausen, Galli, Huber, Aschoff, Huchard, Herrick, Kidd, and others have all cited cases showing extensive disease of the heart muscle due to thrombotic occlusion of a coronary artery where death was postponed for varying lengths of time or did not occur until much later, and then from some other cause.

Herrick, in his recent article, expresses the present view of the results of coronary occlusion as follows:

"While sudden death often does occur, yet at times it is

postponed for several hours or even days, and in some instances a complete, i. e. functionally complete, recovery ensues."

The cases of true embolism reported are few in number. Chiari, Oestreich, Virchow, Rolleston, Hektoen, Welch, MacCallum and Thorel have described instances.

The literature is well covered by Welch in his articles in *Albutt's System of Medicine,* by Thorel (*Ergebnisse der Pathologie,* Lubarsch & Ostertag, 1903) and by Herrick (*Jour. A. M. A.,* Vol. lix, No. 23, December 7, 1912.)

The case which I have to present to-night was in a negro ballplayer, thirty-five years old, who was admitted to the medical service of the Presbyterian Hospital on October 30, 1912. I am indebted to Dr. Janeway for permission to report the case. As a child the patient had rheumatism. Up to two months before admission he had weighed 190 pounds. He had lost gradually until on admission he weighed 157 pounds. This loss of weight, together with loss of strength, constituted his sole trouble until a few days before entering the hospital, when he began to have pain in the right side of his chest, posteriorly.

On admission he was found to be a large, well-built negro, not dyspnoeic or cyanotic. No petechiæ could be found. The lungs were normal except for slight diminution of the breath sounds. The apex impulse of the heart could not be seen or felt. The heart sounds were loudest in the fifth intercostal space, 10 cm. to the left of the mid line The sounds were of fair quality and the action regular. At the apex the first sound was accompanied by a blowing murmur transmitted to the left axilla and upwards along the sternum to the pulmonary area, gradually becoming less marked away from the apex. Just to the left of the sternum, in the third and fourth intercostal spaces, there was also a soft diastolic murmur.

A blood culture taken on admission turned out later to show a growth of what were considered endocarditis cocci.

On the day after admission the patient was perfectly comfortable. At 8 P. M. he was seen and was all right. Ten minutes later he was moaning and complaining of pain in his chest, over

the precordium. He was pale, perspiring profusely, and had a weak pulse. Vomited once. Adrenalin was given. Patient was at this time extremely pale, almost pulseless and complaining of agonizing pain in the back. He was given more stimulation with morphine and a saline infusion was started. It was noted that the vein when exposed was collapsed and practically bloodless. After about 500 cc. of the infusion had been given the patient stopped breathing. His breathing had not been rapid or labored, but in the last half minute it became gasping with increasing intervals.

Autopsy, November 1, 1912. A large framed man, showing considerable loss of weight. No petechiæ found. There was about 100 ccm. of clear fluid in the left chest. The lungs were in full inflation, showing the markings of the ribs over their upper surfaces. Both lungs showed congestion and oedema. The pericardium contained about 100 cc. of clear fluid. The pericardial surfaces were smooth and glistening. Before removing the organs the pulmonary artery was opened and traced to its larger branches without discovering any obstruction.

The heart was not enlarged. The right side presented no abnormalities. The left auricle, ventricle and mitral valve showed no change. No thrombi or post-mortem clots were found in any of the heart cavities. The aortic valve showed the left cusp divided by the incision. The posterior and right cusps were fused into one. The former line of separation could be seen in the Sinus of Valsalva. The opening of the right coronary artery could be seen behind the right portion of this fused cusp. There was a slight amount of yellow atheroma about its mouth but it did not encroach upon the lumen. This large cusp was covered with soft, irregular nodular vegetations, with which was mixed some fresh clot. The vegetations were confined almost entirely to the ventricular surface of the valve, the other surface showing only a few small ones. The lesion as seen upon the left cusp was more destructive. A portion near the right cusp showed no vegetations but was stiff and sclerotic. The rest of the cusp was partially destroyed and replaced by large, irregular, soft masses

of vegetation of dirty pink color. Near the line of junction with the posterior cusp, the vegetations formed a small reddish mass and encroached upon the Sinus of Valsalva. From the summit of this mass there arose a thin pedicle of the same vegetation tissue. It was ½ mm. in diameter and about 6 mm. long. At the end away from the valve it ended in another small nodular vegetation which completely plugged the mouth of the left coronary artery. The opening of the coronary was completely occluded, but the vegetation only extended a short distance into the lumen, the rest of the plug being thrombotic in nature. The plug was pulled out and found to be non-adherent and the wall of the artery smooth. The plug was 1 cm. long, forming a complete cast of the lumen of the vessel. The lower end was branched, fitting into the descending and circumflex branches. There was no change in the heart muscle supplied by these branches.

Infarcts were found in the spleen and kidney.

From the appearance of the plug when pulled out of the coronary it would seem fairly certain that this pedunculated vegetation had swung free in the blood stream for some time and that it had suddenly been swept into the mouth of the left coronary just before the patient's death.

A CASE OF PERIARTERITIS NODOSA.

ALBERT LAMB, M.D.

The patient who forms the basis of this report was a man, age 26. He entered the Presbyterian Hospital, December 7, 1912, on the service of Dr. Janeway to whom I am indebted for permission to use the clinical records of the case. His past history was negative. He had never had syphilis. The only disease which he remembered was an attack of influenza two years before admission. There was nothing in the family history bearing on the case. Two years before he came to the hospital he began to suffer from asthma at infrequent intervals. More recently these attacks have come on every two or three weeks. Three weeks before admission, a nodular swelling the size of an egg appeared quite suddenly in the region of the right ankle. There was intense itching and the surface of the swelling was covered with small purpuric spots. In the course of a few days this swelling disappeared. From this time until he entered the hospital he suffered alternate attacks of asthma and fresh crops of purpura. The latter affected all parts of his body except the face. Often the eruption was accompanied by severe abdominal cramps and pain or by pains in his various points. During his stay in the hospital, the fresh crops of purpura continued to appear with the abdominal and joint pains. He also had attacks of asthma. He ran an irregular temperature. For a few days it would be elevated to 100°-101° and then there would be a few afebrile days. His pulse corresponded with the temperature but was rather more rapid than the height of the temperature would indicate. His leucocytes varied from 17,000 to 21,000 and the eosinophiles from 20 to 51%. His urine always showed albumin, at times up to 0.1-0.2%. There were always hyaline and granular casts and often epithelial and waxy ones. His phthaline excretion was diminished. The Wassermann reaction was negative. About ten days before his death he had an infected ulcer of his leg and just before the end a positive streptococcus blood culture. During the last two days he had a pericardial friction rub. He died February 6, 1913.

At the autopsy, there was found a fresh streptococcus pericarditis, pleurisy and peritonitis. There were two small vegetations on the mitral valve. No organisms were found in these vegetations.

The lungs showed extreme oedema and were in full inflation. The kidneys were slightly enlarged. The capsule was only slightly adherent. On section the cortex was thin, the markings indistinct and the glomeruli appeared as colorless dots. No nodules of any kind were observed on any of the vessels in the ordinary course of the examination. The diagnosis was not suspected until the microscopical examination was made and then on reviewing the material a few tiny nodules were found on the coronary vessels and on section of the kidneys some of the vessels appeared thickened.

On microscopical examination the changes characteristic of Periarteritis Nodosa were found in the vessels of the kidneys, adrenals, stomach, and heart. The lesion as seen without tracing it through serial sections, showed a marked infiltration of the adventitia and surrounding tissue with mononuclear wandering cells and eosinophiles, necrosis and cellular infiltration of the media with the characteristic hyaline appearing band, and rather marked thickening of the intima. In some places, there was considerable new formed connective tissue about the vessels.

The kidneys showed a marked glomerular nephritis with obliteration of most of the glomeruli.

Sections of the vascular lesions stained for bacteria failed to show any.

A full report of the case will be published later when opportunity has been found to examine the serial sections which are being prepared.

DISTRIBUTION OF VITAL STAINS IN ANIMALS WITH INOCULABLE TUMORS.*

ISAAC LEVIN, M.D.

(*From the Department of Pathology of Columbia University, College of Physicians and Surgeons, New York.*)

Vital staining in contradistinction to the usual methods of staining of dead fixed tissue means staining of tissue within a living organism. When a solution of a vital stain is introduced in the organism certain parts of its cells become stained, while the cell itself remains alive and performs normally all its functions, *i.e.*, retains its metabolic activity, its motility and its power of proliferation. Such a method unlike the staining of fixed tissue permits the study of the physiological functions of the cell. R. Heidenhain[1] first applied this method to the study of the functions of the kidneys. But the actual development and practical application of the method is due to the brilliant researches of P. Ehrlich[2] on the subject. His leading idea was that if the chemical constitution of a dye is known and if a certain reaction between the dye and the living tissue produces a color change in the dye, then the nature of the chemical changes which take place in the living tissues could be elucidated.

In 1886 Ehrlich discovered that methylene blue when introduced in the circulation stains blue only certain parts of the nerve tissue. This blue coloring gradually disappears but reappears when the nerve tissue is exposed to the air.

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Ehrlich explains the phenomenon in the following way: The nerve tissue absorbs the oxygen from the methylene blue and transforms it into a colorless body (Leukokörper). The latter then anchors the oxygen from the air and regains its coloring. Further parallel studies with vital stains and drugs led Ehrlich to the conclusion that these dyes as well as drugs when introduced into a living organism may affect one group of cells or organ and leave the other organ unaffected. This specific action of chemical substances on an organism depends on three factors: 1, the chemical constitution of the substance; 2, its pharmocodynamic action; and 3, its specific distribution in the different tissues of the organism.

This close analogy between the action of drugs and dyes led Ehrlich to investigate the therapeutic value of vital stains in diseases which are known to be influenced by drugs. In 1904, he reported in collaboration with K. Shiga[3] successful experiments in the treatment of trypanosome diseases with trypan red. The same diseases are influenced in a far greater degree by arsenicals, but the dye is also of a certain value both alone and in combination with the arsenicals. Nicolle and Mesnil[4] in their studies on trypanosome diseases found that the dyes which are trypanocidal act at the same time as vital stains.

Von Wassermann[5] made an attempt recently to apply the results of the studies on vital staining to therapeutic investigations on inoculable tumors of white mice. He reported on a series of experiments in which he succeeded in curing these tumors with intravenous injection of a chemical combination of selenium and eosin. His experiments were based on the following observation: Gosio[6] had shown previously that natrium selenicum and telluricum are good indications of living cells. While in a mixture of dead cells and one of these salts there forms a precipitation of the metals selenium or tellurium, living cells do not precipitate these metals. In a comparative study of the influence of normal human serums and the serum of cancer patients on cancer cells v. Wassermann used natrium selenicum as an indicator. He noticed that the cells were found dead in

every experiment and concluded that selenium is toxic for cancer cells. An injection of selenium compounds into the circulation of tumor animals failed to influence the growth of the tumors. The failure was due, in his opinion, to the slow penetration of the substance, which allows the normal tissues of the organism to neutralize the selenium. It then occurred to him that in his former experiments on vital staining he observed that eosin and similar fluorescent dyes penetrate very quickly throughout the whole body of the animal upon an intravenous injection. It seemed probable a priori that a combination of eosin with selenium would penetrate a great deal faster into the tumor and consequently increase the efficacy of the selenium. V. Wassermann's experiments upon the therapeutic influence of such a combined substance on mice tumors were very successful as stated above.

Thus recent research indicates that studies on vital staining may become not only of great theoretical but also practical importance. Bouffard[7] was the first to study morphologically the characteristic distribution of vital stains in the organism, but the most painstaking and complete studies on the subjects were done by E. E. Goldmann.[8] He succeeded in developing a technic for the permanent fixation of vitally stained microscopical tissue. He has also shown that there existed a great deal of confusion in the conceptions of the meaning of the term vital staining and clearly defined the conditions. In accordance with his ideas, a vitally staining dye when introduced in the organism intravenously or subcutaneously must remain there a long time, it must not be too toxic for the animal, it must penetrate into the body of the cell and there stain parts of the cell-granules, which are otherwise not visible. Stains which completely answer all these requirements and which were most extensively used by Goldmann in his investigations are trypan blue, isamin blue, and pyrrhol blue. Though of different chemical constitution the three dyes act in an identical manner when introduced into the organism, only

the latter two penetrate the organism much more slowly than the trypan blue. A subcutaneous injection of a solution of any of these dyes produces a diffuse blue staining in the animal. But a microscopical examination of the different tissues shows that the dyes stain only certain granules in specific cells within each organ, as, for instance, the epithelium of the tubuli contorti in the kidney or the Kupfer cells in the liver. The most interesting cell, the granules of which take up the vital stain, is a connective tissue cell found in the skin, mucous and serous membranes and in the sympathetic tissue generally, which Goldmann calls the "pyrrholzelle." It resembles morphologically a large lymphocyte, but is not identical either with a mast cell, plasma cell or a leucocyte. Goldmann considers it to be a histogenous, vitally staining wandering cell with granular protoplasm, which possesses a strong capacity for migration and phagocytic action. These pyrrholcells seem to surround in large numbers any inflammatory focus or other abnormal formation within the organism. In a tubercle, for instance, the pyrrholcells are frequently filled with the tubercle bacilli. Thus the morphological studies of Goldmann indicate as clearly as the chemotherapeutic studies of Ehrlich, and Nicolle and Mesnil, that the vitally staining dyes have a specific distribution in the organism. On the other hand, according to Ehrlich the specific distribution of a chemical is even of greater value in specific therapy than its chemical constitution and pharmacodynamic action.

The great difficulty in influencing or arresting a tumor growth by the aid of chemicals lies in the fact that tumor cells, unlike parasites, are practically identical with normal body cells, and consequently the specific distribution of the chemical into the tumor cells without entering the body cells must be very perfect, otherwise the drug would be as noxious to the rest of the organism as to the tumor. The advantage of the vital stains over any other chemotherapeutic substance lies in the fact that the specific distribution of the former may be controlled morphologically.

In view of this it seemed of importance to the writer to study morphologically the distribution of the vital stains in inoculable tumors of the white rats and mice.

In the first series of experiments a weak aqueous solution of eosin was injected intravenously into mice and rats with inoculable tumors. The animals became nearly immediately intensely colored. But eosin does not seem to be a vital stain in the true meaning of the word. In the first place it is extremely toxic to the animal, furthermore, as was stated by the writer [9] in a preliminary communication, eosin permeates diffusely all tissues, but cannot be detected microscopically anywhere. The same was stated by Goldmann [10] in his latest publication.

It seemed, then, most promising to make use of the same three dyes, trypan blue, isamin blue, and pyrrhol blue, which have given such excellent results in the hands of Goldmann. White rats inoculated with a spindle cell sarcoma or Flexner-Jobling [11] carcinoma were used for these experiments. A subcutaneous injection of two cubic centimeters of a one per cent aqueous solution of the dye was given two or three times at intervals of forty-eight hours. The animals were killed forty-eight hours after the last injection, when the whole body of the animal was deeply stained, the tissues were hardened in ten per cent formalin, then either frozen sections, or, in the case of trypan blue paraffine sections, were prepared and counterstained with alum-carmine. The microscopical examination showed a great deal of difference between the action of the vital stains in the animals with sarcoma and carcinoma, and the two will, therefore, be considered separately.

In the second series of experiments a spindle cell sarcoma was inoculated into white rats, either subcutaneously or into various parenchymatous organs (liver, spleen, kidney or testicle). When the tumors were about two weeks old, the animals were treated with one of the three vital stains mentioned above, they were then killed and the tissues

treated in the manner stated above. The microscopical section of the subcutaneous tumors showed that the connective tissue stroma surrounding the tumor was packed with Goldmann's pyrrholcells. In the tumor itself the majority of the sarcoma cells did not contain any vitally stained granules, but a certain number within the tumor contained the granules. The latter cells did not differ in any perceptible manner morphologically from the rest of the sarcoma cells. The tumors inoculated into parenchymatous organs did not show any surrounding stroma, as the writer [12] stated in previous publications, and there was no conglomeration of pyrrholcells around the tumor. The tumors contained the same quantity of vitally stained cells as the subcutaneous tumors and at the same time the organ tissue surrounding the growing tumor contained the same number of vitally stained specific cells as the normal organ. Figure 1 shows an inoculation of the sarcoma in the kidney. The tumor contains a few vitally stained cells, which cannot be distinguished morphologically from the other non-stained sarcoma cells. The kidney tissue shows the vitally stained epithelial cells of the tubuli contorti and in no way differs from vitally stained normal kidney tissue.

In the third series of experiments Flexner-Jobling adenocarcinoma was inoculated into white rats either subcutaneously or into various parenchymatous organs. The microscopical section of the subcutaneous tumors showed that the connective tissue stroma surrounding the tumor was packed with pyrrholcells as was the case in the sarcoma inoculated subcutaneously, but the tumor itself did not contain any vitally stained cells. Figure 2 shows this condition in a subcutaneous tumor. Tumors inoculated into parenchymatous organs do not show any surrounding stroma formation with pyrrholcells nor can vitally stained cells be found anywhere between the tumor cells. The conditions of vital staining of the organ cells surrounding the tumor are identical with the conditions of the same organ in which the tumor was not inoculated.

The results of this investigation seem to indicate that the vital stains when introduced into an animal with an inoculable tumor do not show any specific distribution which would show a predilection for the cells of the inoculated tumors. The organ cells surrounding the tumor contain the normal quantity of the vitally stained cells, while the tumor itself either contains none or very few such cells. It is of interest, though, to note that of the two tumors used in this investigation the carcinoma did not show any of the vital stains while sarcoma showed a certain amount. It is possible, then, that a chemical may influence one kind of tumor and not influence another.

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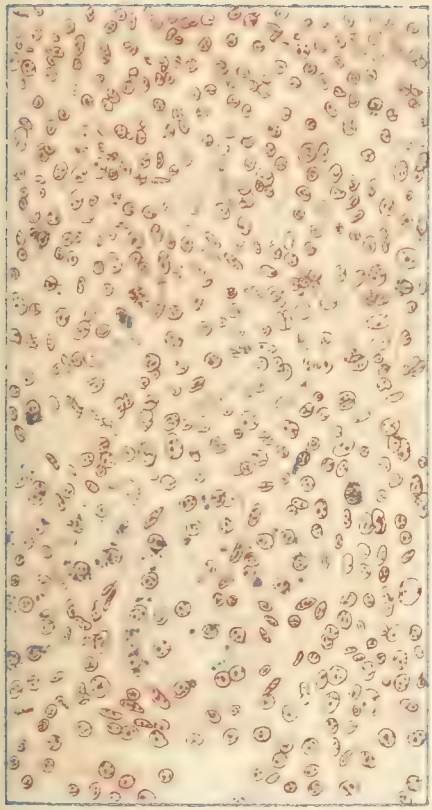
EXPLANATION OF PLATE I.

FIG. 1. — Sarcoma inoculated in the kidney. Trypan blue. Alum carmine — high power.

FIG. 2. — Flexner-Jobling carcinoma inoculated subcutaneously. Trypan blue. Alum carmine — low power.



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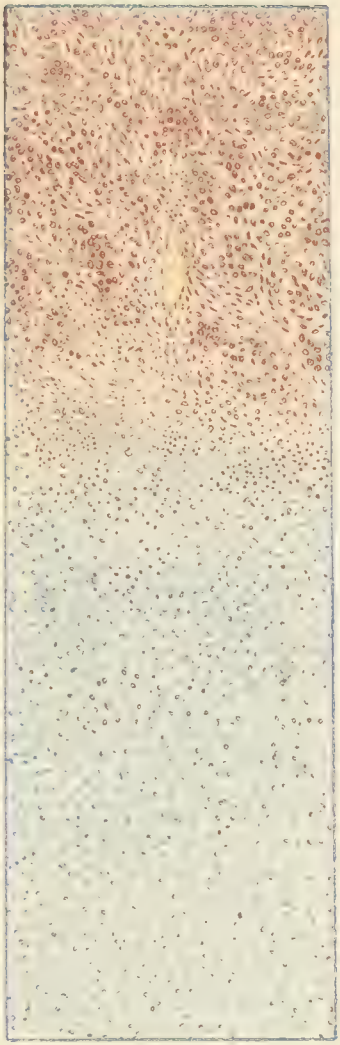

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Vital staining.

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THE MECHANISMS OF METASTASIS FORMATION IN EXPERIMENTAL CANCER.*

BY ISAAC LEVIN, M.D.

(From the Department of Pathology of Columbia University, College of Physicians and Surgeons, New York.)

PLATES 25 AND 26.

The term metastasis was originally used to indicate the transportation of any substance, whether metal or coal dust, normal cells (placenta, bone marrow), bacteria, or cancer cells from one part of the organism to another. von Recklinghausen (1) was the first to restrict the meaning of the term and used it only in connection with the transportation of pathological substances. More recently the word metastasis has been used only in connection with the secondary tumor nodules which occur so frequently in malignant growths. This restricted use of the term was caused by the generally recognized fundamental difference which exists between a multiple granuloma of an infectious disease and a true metastatic growth of a malignant tumor. While in the former only the bacteria or the noxious agents are transported into the distant parts of the organism where the granuloma is formed from the local cells, a metastatic tumor growth originates in every case through the proliferation of a group of cancer cells detached and transported from the primary tumor.

The mechanism of the formation of metastasis in malignant growths consists of the following phases: (1) the detachment of a group of cancer cells from the primary tumor; (2) the transportation of this group of cells through the blood or lymph channels into distant parts of the organism; and (3) the proliferation of these cells in the new location and the consequent formation of metastatic tumors. It is well known that both the frequency of the

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occurrence of metastases and the localization of the metastatic growths varies widely in different malignant tumors.

Most pathologists (von Recklinghausen (2), Lubarsch (3), and Schmidt (4)) consider the difference in the channels of transportation to be the main reason for the difference in the frequency and localization of the metastatic tumors. The causes are purely mechanical, namely, the differences in the morphological structure of the organs, the relative size of the cancer cells, the capillaries of a certain organ, and so on.

More recently Albrecht (5) and Ehrlich (6) advanced the hypothesis that the success of the development of a metastatic tumor is due to the chemical metabolic conditions of the entire organism. Their idea is that the growth of the secondary tumor depends upon the character of the primary growth. If the latter is very malignant and reaches a large size, then it uses up all the specific food required by the multiplying cancer cells, and none of it remains for the cells of the secondary growth. On the other hand, when the primary tumor is growing slowly, a sufficient amount of the specific food is left for the development of the metastatic tumor. Recent investigations of the writer (7) indicate that neither the purely mechanical conceptions nor the athreptic theory of Ehrlich are adequate to explain all the phenomena of the formation of metastasis.

The investigations were conducted mainly on two inoculable tumors of the white rat,—a spindle cell sarcoma which was studied originally by Jensen, and an adenocarcinoma which was described by Flexner and Jobling (8). The sarcoma is very malignant, grows to a large size, and apparently, in accordance with Ehrlich's theory, does not form any metastases after a subcutaneous inoculation. When an emulsion of this tumor was injected intravenously into a healthy animal no tumor growth formed anywhere. In these experiments lack of specific food could not play any part since the injected animals had no primary tumor. On the other hand, when the same tumor was inoculated into a parenchymatous organ, the spleen or the liver, metastases formed in a certain number of cases.

The second tumor, the Flexner-Jobling adenocarcinoma of the white rat, induces the formation of metastases when inoculated subcutaneously. An intravenous injection of an emulsion of this tumor induced the formation of metastatic nodules in parenchymatous organs.

In the two series of experiments the conditions within the host, both as regards the mechanical structure of the organs as well as nutrition, were identical; all the animals were normal. The

methods of experimentation were also identical and the only difference consisted in the different character of the cancer cells The sarcoma cells injected into the circulation did not develop into a metastatic growth, while the adenocarcinoma cells did. The formation of metastasis upon the inoculation of the sarcoma into the liver and spleen was apparently due to the change of character which the sarcoma cells underwent during their sojourn in the parenchymatous organs. Such changes in the character of the cells after a lodgment in a different organ may occur also in human pathology, as was shown by Hansemann (9) and others.

In the present investigation the writer undertook to study the influence of the organism of the host upon the development of secondary metastatic tumors, while the character of the cancer cells used for the experiments was not changed. The research was conducted with the same two tumors of the white rat described above.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT INOCULATION INTO A PARENCHYMATOUS ORGAN.

In a previous investigation (10) it was shown, in agreement with other investigators, that it is possible to inoculate successfully under the skin a tumor in an animal bearing in a different region a previously inoculated subcutaneous tumor. In order to approach more nearly the conditions obtained during the formation of metastasis, inoculations were made into a parenchymatous organ of animals in which a subcutaneous inoculation was done previously. In a former investigation the writer (11) has shown that when an animal is resistant to a subcutaneous inoculation of a tumor it will also resist in every case a subsequent inoculation into an organ. In the present study an inoculation was done into parenchymatous organs of animals of a tumor which was previously inoculated into the same animal subcutaneously.

With regard to the method used in the experiments the following explanation should be made. A normal rat, as has been shown by the writer in a number of investigations, stands a laparotomy and inoculation of a tumor into an organ very well and there is practically no postoperative mortality. But tumor animals are a

great deal more vulnerable, and a number of them succumb to the operation. A description of the experiments follows.

Experiment 1.[1] *Sarcoma in the Liver.*—A laparotomy was done on eighteen rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced by the aid of a trocar needle into the liver. Of the thirteen animals that survived the operation for two weeks, six developed a large subcutaneous tumor and also a fairly large sized tumor in the liver. Figure 1 shows one of these animals. Seven animals developed only a small nodule subcutaneously and none of them developed any growth in the liver.

Experiment 2. Carcinoma in the Liver.—An inoculation of the adenocarcinoma into the liver was done on twenty-six rats which were previously inoculated subcutaneously with the same tumor. Sixteen animals survived the operation and of these five developed a large subcutaneous tumor and also a fairly large tumor in the liver. Eleven animals developed a small subcutaneous nodule and showed no growths in the liver.

Experiment 3. Sarcoma in the Spleen.—A laparotomy was done on nine rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced into the spleen by the aid of a trocar needle. Of the six animals that survived the operation for two weeks, three developed a large subcutaneous tumor and a fairly large tumor in the spleen Three animals developed a small nodule subcutaneously and showed no growth in the spleen.

Experiment 4. Carcinoma in the Spleen. The same operative method was employed as in experiment 3. The carcinoma was inoculated in the spleen of six rats in which a subcutaneous inoculation of the same tumor was previously done. The three rats that survived the operation for two weeks developed a large subcutaneous tumor and a fairly large tumor in the spleen.

The results of the series of experiments with subcutaneous inoculations and subsequent inoculations into a parenchymatous organ are significant inasmuch as they clearly indicate the influence of the organism of the host on the growth of metastases. The subsequent inoculation into an organ of a tumor-bearing animal represents an artificially induced formation of a metastasis. The results of the present as well as the former investigations of the writer show that an inoculation into an organ will not succeed in an animal into which a previously attempted subcutaneous inoculation failed entirely or in which there developed only a small subcutaneous nodule. On the other hand, in the animals in which the subcutaneous inoculation produced the formation of a large subcutane-

[1] All the experiments were done under ether anesthesia.

ous tumor, the subsequent inoculation into an organ was also invariably successful. It is a well established fact that the success or failure of growth of a primary tumor inoculated in a normal animal is due to the conditions of general susceptibility or resistance of the organism of the animal to the growth of the tumor. Apparently the same general phenomena influence the growth of the secondary inoculated tumor, or, in other words, influence the formation of the artificial metastases.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT SIMULTANEOUS INOCULATION INTO TWO PARENCHYMATOUS ORGANS.

The formation of metastases is a complex phenomenon. Not only does the frequency and localization of metastatic growth differ in the various tumors, but even the same tumor may form metastases in different organs in different individuals. In order to imitate experimentally this phase in metastasis formation, a simultaneous inoculation was done into two parenchymatous organs.

Experiment 5. Sarcoma in the Liver and Spleen.—The same operative methods were employed as in the previous experiments. A laparotomy was performed on sixteen rats which received two weeks previously a subcutaneous inoculation of the sarcoma. A small piece of the tumor was introduced subcutaneously into the liver and the spleen. Of the fifteen animals that survived the operation for two weeks, six developed only a small subcutaneous nodule and in these there was found no growth either in the liver or in the spleen. Nine animals developed large subcutaneous tumors, and of these, three showed a growth only in the liver, and three showed growth only in the spleen.

The results of this series of experiments show that, on the whole, the results of the multiple inoculations also depend upon the general conditions of resistance or susceptibility of the organism of the host. When the tumor fails to grow it fails everywhere, and when it succeeds, it succeeds in every organ. On the other hand, in those instances where the tumor grew both subcutaneously and in one organ and failed to grow in another organ there must have been an additional factor; namely, while the organism of the animal as a whole was susceptible to the inoculation both subcutaneously and into one organ, the other organ, the spleen or the liver, resisted the growth of the tumor. The possibility of such a local organ resistance in an animal of which the general organism is

susceptible to the growth of the same tumor was proven by the writer (12) in a recent investigation.

The Flexner-Jobling carcinoma of the white rat grows readily when inoculated anywhere in the organism, but fails to grow when inoculated in a normal testicle. But when an inflammatory lesion is produced in the testicle previous to the inoculation of the tumor, then the tumor inoculation is successful. The mechanism apparently causes the failure of growth of a tumor in one organ, while the same tumor grows subcutaneously and also in another organ.

SURGICAL REMOVAL OF A SUBCUTANEOUS TUMOR AND SUBSEQUENT INOCULATION INTO PARENCHYMATOUS ORGANS.

Uhlenhuth, Händel, and Steffenhagen (13) have shown recently that when a subcutaneous tumor is removed surgically and the same tumor inoculated subcutaneously in a different place, the second inoculation will not succeed if the removal of the first tumor was radical; but when there is a recurrence after the removal of the first tumor, then the second inoculation also succeeds. These striking results were confirmed by Meidner (14), Händel and Schönburg (15), and Uhlenhuth, Dold, and Bindseil (16). Apolant (17) seems to have reached different results and claims that there is no relation between the recurrence of the first tumor and the success of the second inoculation.

The influence of the surgical removal of a primary tumor upon the growth of metastases is of great practical and theoretical importance. In view of this and also of the contradictory results of the work of the different investigators it seemed advisable to repeat and enlarge upon these experiments by removing surgically the subcutaneous tumors and subsequently inoculating the same tumor into one or two parenchymatous organs.

Experiment 6. Carcinoma Removal and Inoculation in the Liver.—In ten animals a longitudinal incision was made at the inner side of the subcutaneous tumor and the latter was enucleated *en masse*. Immediately following this step the peritoneum was opened and a small piece of the animal's own tumor was inoculated into the liver. Of the five animals that survived the operation the primary tumor recurred in three, and in these there was a positive growth in the liver; in two animals there was no subcutaneous recurrence and no growth in the liver.

Experiment 7. Sarcoma Removal and Inoculation into the Liver and Spleen.—The method of operation was identical to the one described in experiment 6. In eleven animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Of the ten animals that survived the operation, in seven there was no recurrence of the subcutaneous tumor and in these the inoculation into the organs failed invariably. In three animals the subcutaneous tumor recurred and of these animals in one the growth was positive in both liver and spleen (figure 2), in one growth was found only in the liver, and in the last animal a growth was found in the spleen.

Experiment 8. Carcinoma Removal and Inoculation into the Liver and Spleen.—The method of operation was the same as in the previous series of experiments. In twenty-three animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Seventeen animals survived the operation and in eight of these there was neither a recurrence of the subcutaneous tumor nor any growth in the organs. In three animals there took place a recurrence of the subcutaneous tumor and positive growth both in the liver and the spleen. In the last six animals there was a recurrence of the subcutaneous tumor and a positive growth only in the spleen.

This series of experiments in the first place corroborates the findings of Uhlenhuth and his collaborators, that a subsequent inoculation succeeds whenever there is a recurrence of the primary tumor, and fails when the removal of the primary tumor remains radical. The experiments show further, like the previous experiments of the present study, the influence of the general and local resistance or susceptibility of the organism of the host on the growth of the secondary metastatic tumors. They also emphasize the relation between the postoperative recurrence of the primary tumor and the formation of metastasis. When the organism possesses a certain amount of resistance it will neutralize the few cancer cells which must necessarily be left behind after the apparently most radical operation; there will not take place any local recurrence and for the same reason the secondary or metastatic tumor will fail to grow. While, on the other hand, when this resistance of the organism is lacking, notwithstanding the most painstaking operation both a local recurrence and the formation of metastasis will take place.

The greater frequency of the growth of carcinoma in the spleen than in the liver may be due to the greater vulnerability of the former organ. The spleen of animals inoculated with the car-

cinoma frequently shows the condition of an amyloid degeneration, and, as has been shown by the writer in experiments with the testicle, a diseased organ is more susceptible to growth of tumor than a normal one. The writer is at present engaged in the study of this question.

The analysis of the results of the present investigation shows clearly that the differences in the frequency of the occurrence and in the specific localization of metastases are not due to the ease of detachment of the cancer cells from the primary tumor. Nor does the difference in the mode of transportation play a part. The main factors in determining the localization and frequency of metastases are the character and malignancy of the cancer cell on the one hand, and the general and local susceptibility of the organism of the host on the other. The failure or success of the proliferation of a group of cancer cells transported from the primary tumor into a distant organ is a result of the interaction of these two causes.

The validity of this conclusion will be further enhanced when it is considered that the mechanism and cause of growth of the primary tumor and the metastasis are identical. In inoculable tumors this identity is certainly complete since even the primary tumor is caused by the proliferation of a group of cancer cells introduced from outside. But even in a spontaneous tumor of the same animal, or in human cancer, the difference between a primary tumor and a metastasis consists only in the fact that in the former one must consider the additional unknown factor, namely, the transformation of a normal cell into a cancerous one. Otherwise the further growth and development of the primary tumor, as well as the metastasis, depends upon the correlation of the two factors mentioned above.

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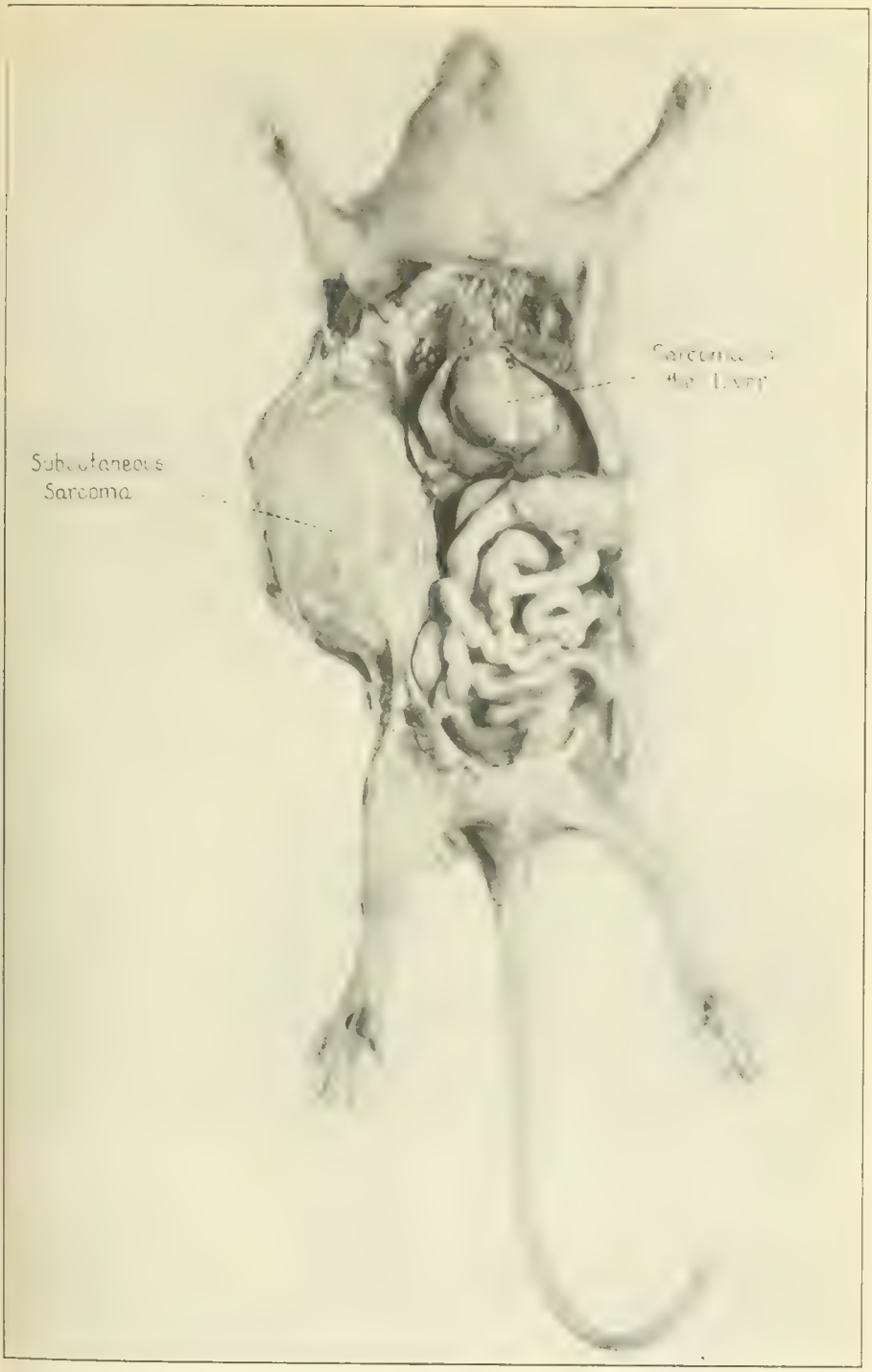

FIG. 1.

(Levin: Metastasis Formation in Experimental Cancer.)

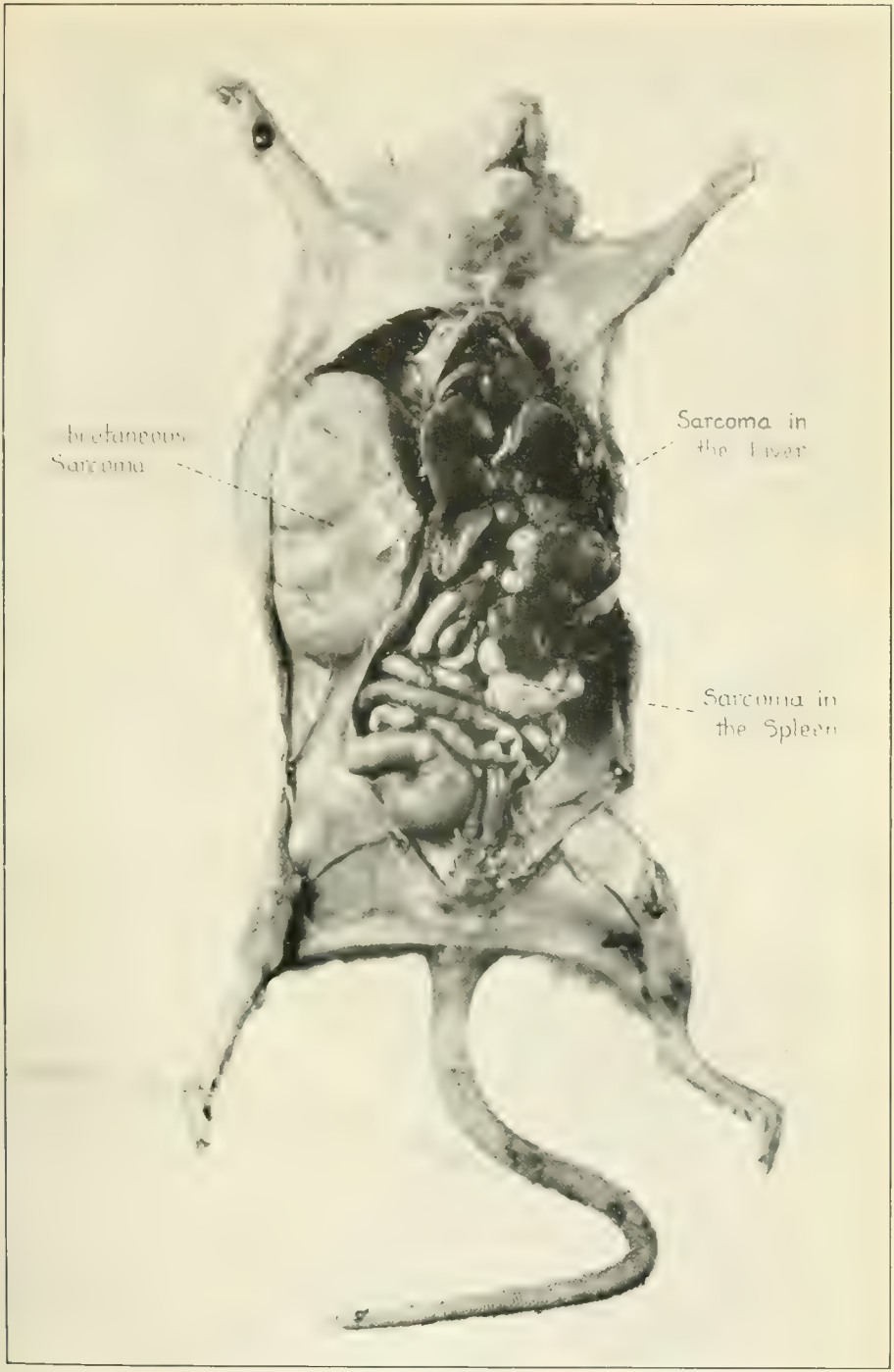

FIG. 2.

(Levin: Metastasis Formation in Experimental Cancer.)

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EXPLANATION OF PLATES.

Plate 25.

Fig. 1. Sarcoma of the white rat. A large subcutaneous tumor and a fairly large tumor in the liver.

Plate 26.

Fig. 2. Sarcoma of the white rat. A large subcutaneous tumor and a tumor growth in the liver and in the spleen.

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CONGENITAL VARIATIONS IN THE PERITONEAL RELATIONS OF THE ASCENDING COLON, CÆCUM AND TERMINAL ILEUM

S. C. HARVEY, M.D.

(*From the Department of Pathology of the College of Physicians and Surgeons*)

In the last five years increasing attention has been directed to certain signs and symptoms in the right side of the abdomen which have been usually interpreted as indicative of chronic appendicitis. Such conditions are the "cæcum mobile" of Wilms (1), the ileal band of Lane (2) and the "membranous pericolitis" of Jackson (3). The data as regards frequency of occurrence of these conditions has been in great part derived from instances seen at operation. Likewise the etiology of the adhesions about the colon and ileum has been dependent upon this source of information and in consequence there has arisen

two theories as to their origin. The one, as represented by Pilcher (4), Gerster (5) and others, regards them as formed by a previous subacute inflammation, the other, as supported by C. H. Mayo (6), Flint (7), Reid (8) and others, asserts them to be but developmental defects. The examination of fœtuses, notably by Flint, has given greater weight to the later hypothesis.

In order to obtain a larger amount of data bearing upon the frequency of occurrence of these abnormalities and their mode of origin, 105 children varying in age from birth to three years and seven months were examined post mortem at the New York Foundling Hospital. Of these 43 were in the first two months of life, 28 between three and seven months of age and 24, seven months old or over.

The conditions looked for were: abnormal mobility and position of the cæcum, undue development of the cysto-colic fold, abnormal omental attachments, pericolic membranes, pericæcal membranes, appendicular membranes, and Lane's band. The frequency of occurrence was as follows:

	Number Found.	Per Cent.
Cæcal displacement	32	30
Cysto-colic fold	20	19
Omental attachments	10	9
Pericolic membranes	17	16
Pericæcal membranes	13	12
Appendicular membranes	8	8
Lane's bands	9	9

Of these abnormalities 15, or 14 per cent., were sufficient to cause some angulation or kinking of the colon, appendix or ileum. The variations in the position of the cæcum as well as of the cysto-colic fold were of the same frequency in those under three months as in those over this age. The membranous structures, however, seemed rather to decrease with the age, and this was especially true of Lane's band. As regards sex, abnormalities in the position of the cæcum were more frequent in the female and this was also true of Lane's band. On the other hand, the remaining membranes were slightly more prevalent in the male.

From a consideration of the above data one must conclude

that these peritoneal abnormalities about the ileum and the ascending colon are of frequent occurrence and are developmental in origin.

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Discussion:

Dr. Hillman: We have had between 50 and 60 autopsies on infants and young children at the Post-Graduate Hospital during the past two years and I have been looking for adventitious peritoneal bands and have found in probably one third of the cases membranes which in the adult would be classified as abnormal peritoneal structures. Looking at the problem from the point of view of both anatomy and pathology, it would seem that in these very young children such a comparatively high percentage of cases in which these membranes are found would rather suggest that the affair is of a developmental character, and not inflammatory, as there are no other evidences of inflammation in the peritoneal cavity. Pericolic membranes, Lane's bands, or membranes about the hepatic flexure and elsewhere are often accompanied, in these children, by extremely lax mesenteric attachments, so that you have a loose mesocolon running from the cæcum right around to the rectum. In cases where the child has been marasmic we have noticed that this redundant condition of the mesocolon is marked. Whether the adventitious membranes are related to the laxity of the peritoneal ligaments I do not know, but it has struck me that these bands are of a developmental rather than of an inflammatory type.

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ON THE PHOSPHORUS CONTENT OF THE BLOOD OF NORMAL AND PARATHYROIDECTOMIZED DOGS.

By ISIDOR GREENWALD.

(From the Laboratories of Pathology and of Biological Chemistry of Columbia University and the Chemical Laboratory of the Montefiore Home.)

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As a result of previous experiments upon dogs,[1] in the course of which a marked retention of phosphorus was observed after removal of the parathyroid glands, it became of interest to ascertain the form in which phosphorus was stored in the organism under the experimental conditions. It is generally conceded that the tetany consequent upon parathyroidectomy strongly suggests an intoxication by a circulating toxin. In these experiments, therefore, the amount and distribution of phosphorus in blood and serum was investigated.

The experiments were conducted in pairs. After a few days' observation, during which period the dog was kept on a constant, weighed diet,[2] the parathyroids were removed. As soon as the twitching became well marked, the animal was exsanguinated. In the earlier experiments this was accomplished under ether anesthesia and from a carotid artery. Later, the dogs were bled from a femoral artery, using cocaine. The control dog, of the same sex (except in some of the earlier experiments), and of nearly the same size and age, was fed for a number of days on a diet that was, per kilo, the same as that of the experimental animal and was bled at the same interval after the last meal as was the parathyroidectomized dog. In this way it was hoped to eliminate the effect of age, sex, the absorption of food and of long-continued tetany.[3]

[1] Greenwald: *Amer. Journ. of Physiol.*, xxviii, p. 103, 1911.

[2] This never contained bone-ash. Infusorial earth was used in its stead.

[3] For further details as to the conduct of the experiments see Greenwald: *loc. cit.*

For the estimation of phosphorus a slight modification of the Neumann[4] method was used. It was found that, if Neumann's directions were followed, precipitation of the phosphomolybdate was not always complete. If, however, after oxidation was complete, the acid was diluted and neutralized with ammonium hydroxide, then acidified with nitric acid, heated to about 65°, complete precipitation occurred on addition of ordinary acid molybdate solution and digestion at 65° for one or two hours. The mixture was then allowed to stand at room temperature for several hours, ordinarily over night, then filtered on a Gooch crucible fitted with a piece of hardened filter paper and washed with cold water. The crucible and the precipitate were placed in the beaker in which the precipitation had been made and the precipitate was dissolved in a slight excess of standard sodium hydroxide solution (approximately seventh-normal). From 10 to 30 cc. of formalin,[5] previously neutralized to phenolphthalein with sodium hydroxide, were added, then 10 or 25 cc. of standard acid and finally standard sodium hydroxide solution until the liquid was faintly alkaline to phenolphthalein. The volume of the alkaline solution required, less the volume of the acid used, multiplied by the factor, gave the amount of phosphorus in the sample. Even with less than 1 mgm. of phosphorus the error is only ±2 per cent.

For the estimation of the different forms of phosphorus in blood and serum, several methods were tried. Most of these were found to be unsuitable. At first the blood was received in tared flasks containing glass beads and thoroughly shaken. The flasks were weighed and the contents transferred to a bottle containing several volumes of 95 per cent alcohol, recently distilled over sodium hydroxide. After standing a few days, the mixture was filtered through a linen bag and the residue extracted with hot alcohol in a continuous extraction apparatus. It was found that the extracts were always deeply colored and that they contained considerable quantities of material (hematin?) which made subsequent separation of the lipoids, either by precipitation with chloroform[6] or by extraction with anhydrous ether, difficult and inaccurate.

[4] Neumann: *Zeitschr. f. physiol. Chem.*, xxxvii, p. 115, 1903.
[5] Bang: *Biochem. Zeitschr.*, xxxii, p. 443, 1911.
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An attempt was made to dry the blood by mixing it with infusorial earth to form a thick paste and exposing this to the air, or *in vacuo*, at a moderate temperature (30–35°). A fine, dry powder was readily obtained but it was found that extraction with the usual solvent, removed only a small portion of the phospholipins known to be present.

For precipitation of the proteins and extraction of the lipoids, acetone was found to be most suitable. Addition of four volumes of acetone to blood or serum precipitates protein and inorganic phosphates completely. Subsequent treatment of the precipitate with hot acetone removes the lipoids practically completely but does not affect hemoglobin nor dissolve inorganic phosphate. If the acetone extract be evaporated and the lipoids precipitated with chloroform, only traces of phosphorus are found in the filtrate. After extraction with acetone, treatment with hot alcohol and ether removes, at most, only traces of phosphorus compounds from serum and only comparatively small amounts from whole blood.

For the determination of water-soluble phosphorus, direct extraction of the residue from the lipoid extraction with hot water and dialysis were tried. Both were failures, removal of inorganic phosphate being incomplete within any reasonable period. Extraction with dilute hydrochloric acid was also unsuccessful, for the protein swelled to a jelly. Direct estimation of the non-colloidal phosphorus of serum was attempted in the filtrate from the kaolin precipitate produced by the method of Michaelis and Rona,[7] but it was found that all of the inorganic phosphate added could not be recovered. Apparently some had been adsorbed.

The procedures that give the best results in the estimation of water-soluble phosphorus are the following:

1. The serum or blood is mixed with nine or nineteen volumes, respectively, of a solution containing 1 per cent of acetic acid and 0.5 per cent of picric acid. After a few hours the liquid is filtered and the phosphorus in an aliquot portion of the filtrate is estimated in the usual manner.

2. The dry residue from the lipoid extraction is ground to a powder and treated with a dilute solution of hydrochloric acid (1

[7] Michaelis and Rona: *Biochem. Zeitschr.*, vii, p. 329; viii, p. 356, 1908.

per cent by volume of the concentrated acid) saturated with picric acid. Ten or twenty volumes, respectively, of this solution are used for each volume of serum or blood under examination. The mixture is shaken at intervals and allowed to stand over night. It is then filtered and phosphorus is estimated in an aliquot portion of the filtrate.

Concordant values for the phosphorus precipitated from these extracts by barium and ammonium hydroxides were not obtained; consequently only total phosphorus was estimated.

That these methods are fairly accurate is shown by the figures given in tables I–III.

As already stated, separation of the phosphorus compounds of the blood into various fractions was not successful in the first series of experiments. The figures given in table IV were obtained by adding together the values obtained for the phosphorus in the different fractions. In the case of dog 111, however, the total phosphorus was estimated directly. As can readily be seen, the phosphorus content of the blood of the parathyroidectomized dogs was greater than in that of the control animals.

In the two following experiments, the blood was mixed with infusorial earth and dried. On subsequent extraction, phospholipins were not removed by the usual solvents. However, determinations of the total solids, nitrogen and phosphorus were made with the results indicated in table V. Six months later, samples of this dry powder were extracted with the picric-hydrochloric acid mixture and phosphorus estimated in the extract. The values obtained are given in the table.

The material for the next series of analyses consisted of defibrinated blood from a normal and a parathyroidectomized dog, obtained in the course of other experiments. Data relating to the sex, weight and food of the dogs are lacking. In this series the acetone extraction method was used but the residues were wasted in a vain attempt to remove inorganic phosphate by dialysis or by means of boiling water. However, direct estimations of the acid-extractable phosphorus were made, using the acetic acid-picric acid mixture already described. The figures obtained are given in table VI.

The blood from the next pair of dogs was allowed to clot. The serum was centrifuged to remove all the cells and then analyzed. Unfortunately, scarcity of material and a series of accidents made

it impossible to determine more than the total nitrogen and phosphorus and the phosphorus extracted by acetone and by subsequent treatment with absolute alcohol and ether. The figures obtained are presented in table VII.

Because parathyroidectomy was not followed by symptoms of parathyroid insufficiency, the next dog, 214, had both thyroids removed. Tetany then developed and the dog was bled. The blood, as also that of the control, was oxalated. In calculating the results of the analyses, which are summarized in table VIII, proper allowance has been made for the weight of the ammonium oxalate solution used.

In the three following experiments, perfectly clear serum, almost free from hemoglobin, was obtained. If the serum is at all red, it contains enough inorganic phosphate derived from erythrocytes to make analysis useless. The results obtained are summarized in table IX.

As can readily be seen on examining the tables, the total amount of phosphorus in the blood and serum of parathyroidectomized dogs is regularly greater than in normal dogs. This increase is not associated with a corresponding increase in the amount of total solids or of nitrogen; on the contrary it may be very marked in the presence of an equally pronounced diminution of total solids and nitrogen. The increase seems to be due largely, if not entirely, to an increase in that form of phosphorus which, for want of a better name, we have called acid-extracted phosphorus. A part of this fraction is inorganic phosphate; whether or not all of it is inorganic, cannot be stated. The amount of lipin-phosphorus in the blood and serum of dogs varies considerably and, apparently, bears no relation to the presence or absence of the parathyroid glands.

Very recently Juschtschenko[8] has published the results of an investigation into the nitrogen and phosphorus content of the tissues of normal and parathyroidectomized dogs. Only his results with serum need concern us now. He dried the serum, first at 30°, then at 65°, and estimated inorganic phosphate by the method of Stutzer and Neumann. He states that the amount of inorganic phosphate of the serum is the same in dogs in tetany and in normal dogs. He makes no reference whatever to his

[8] Juschtschenko: *Biochem. Zeitschr.*, xl, p. 64, 1912.

earlier paper[9] in which he claimed that the blood of dogs and rabbits in tetany after thyroidectomy contained more inorganic phosphate than did the blood of normal animals. It is also interesting to note that, in the two instances in which he determined the phosphorus in the residue obtained after extraction of the lipoids from the dried serum, the higher values were obtained in the preparations from parathyroidectomized dogs. The conclusions drawn by Juschtschenko from his experiments are open to other objections. His dogs were in tetany for hours and days before they were bled so that it is impossible to distinguish the effect of the parathyroidectomy from the possible effect of the tetany and inanition. Phospholipins are apt to decompose when kept at 65° for twenty-four hours, as occurred in Juschtschenko's method of drying the serum. Apparently he did not attempt to test the accuracy of his method, to see if inorganic phosphate added to the serum could be recovered.

SUMMARY.

After removal of the parathyroid glands, the total phosphorus of the blood and serum of dogs is increased. This may be observed at a time when the tremor is still very slight. The increase may be as much as 160 mgm. of phosphorus per kilo of blood. The greater part of this increase is in the fraction which is insoluble in the usual lipoid solvents but is soluble in a mixture of dilute hydrochloric or acetic and picric acids.

It is with great pleasure that I acknowledge my indebtedness to Dr. W. G. MacCallum, who was kind enough to remove the parathyroids from the animals required.

TABLE I.

Acid-extractable phosphorus in 25 cc. of ox-blood.

(Results expressed as cubic centimeters of standard solution required for titration of the phosphomolybdate.)

EXTRACTED WITH DILUTE ACETIC AND PICRIC ACIDS	EXTRACTED WITH HYDROCHLORIC AND PICRIC ACIDS, AFTER EXTRACTION WITH ACETONE
cc.	*cc.*
20.45	20.40
20.50	20.50
	20.50

[9] Juschtschenko: *Zeitschr. f. physiol. Chem.*, lxxv, p. 141, 1911.

TABLE II.

Recovery, by the picric-acetic acid method, of inorganic phosphate added to blood.

($Mg_2P_2O_7$ dissolved in dilute HNO_3, neutralized with NaOH before adding to the blood. Results expressed as cubic centimeters of standard solution required for titration of the phosphomolybdate.)

NUMBER	BLOOD	ADDED PHOSPHATE	BLOOD AND PHOSPHATE	PHOSPHATE RECOVERED
	cc.	*cc.*	*cc.*	*cc.*
1	15.17	49.85	64.00	48.83
	15.17			
2	20.45	49.85	70.50	50.02
	20.50	49.85	70.70	50.22
		49.85	70.10	49.62
3	26.4	66.0	92.2	65.8
	26.2	66.0	92.0	65.8

TABLE III.

Recovery, by the picric-hydrochloric acid method, of inorganic phosphate added to blood.

($Mg_2P_2O_7$ dissolved in dilute HNO_3, neutralized with NaOH before adding to blood. Results expressed as cubic centimeters of standard solution required for titration of the phosphomolybdate.)

ACETONE-SOLUBLE PHOSPHORUS		ADDED PHOSPHATE	ACID-EXTRACTED PHOSPHORUS		PHOSPHATE RECOVERED
Blood	Blood and phosphate		Blood	Blood and phosphate	
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc*
11.45	11.60	49.95	20.40	69.60	49.20
	11.30	49.85	20.50	70.45	49.95
		49.85	20.50	71.33	50.83

TABLE IV.

Total phosphorus in blood from normal and parathyroidectomized dogs.

NUMBER	PARATHYROIDECTOMIZED	NUMBER	CONTROL
	mgm. per kilo		*mgm. per kilo*
105	477		
107	521	108	425*
110	512	109	416
111	474†		

* Parathyroids removed ten days before bleeding; no symptoms.
† Chronic latent tetany. See protocol of experiment below.

Dog 105. Fox-terrier, male, weight 10.70 kilos. The parathyroids were removed at 10 a.m., January 19, 1911. On the following day, slight twitching was noticed at 2 p.m. This was more marked at 3 p.m., when the dog was exsanguinated.

Dog 107. Mongrel, bitch, weight 9.20 kilos. Parathyroidectomy was performed at 2 p.m., January 31, 1911. Slight twitching was noticed at 2.30 p.m., February 4, and at 3.30 the dog was bled.

Dog 108. Mongrel, male, weight 8.35 kilo. Four parathyroids were removed on February 4, 1911. No symptoms appeared and the dog was bled at 3 p.m., February 14.

Dog 109. Bull-terrier, bitch, weight 8.30 kilos. Exsanguinated at 4.30 p.m., February 14, 1911.

Dog 110. Beagle, bitch, weight 9.60 kilos. Parathyroidectomy was performed at 3 p.m., February 10, 1911. Very slight twitching was noticed at 3.30 p.m., February 12. This gradually became more marked until, at 5.30 p.m., the dog was exsanguinated.

Dog 111. Bull-terrier, bitch, weight 13.0 kilos. Parathyroidectomy was performed on March 21, 1911, but no symptoms of parathyroid insufficiency appeared within the following two weeks. On April 6, a pup was born prematurely and died the same day. Another was born the following morning and lived about twenty-four hours. On the morning of April 8, a slight tremor was noticed. This grew more marked and continued several days. Two pups were found in the cage on the morning of April 10. They were quite well developed but the mother paid little attention to them and they died the following morning. On this day (April 11), four pups made their appearance. The mother suckled them but one died on the 16th and another on the 17th. The other two survived and were raised to an age of about four months when they were unfortunately lost. They seemed to be quite normal. The mother continued in tremor until April 21, two weeks in all. In June, attempts were made to induce tetany by feeding large quantities of meat and of extract of beef. These were not successful. After a return to the usual diet for three days, the dog was bled at 3 p.m.

TABLE V.

Analyses of blood from a parathyroidectomized dog (209) and from a normal dog (210).

	209	210
Total solids	17.80 per cent.	24.26 per cent.
Total nitrogen	2.31 per cent.	3.16 per cent.
Total phosphorus	592.7 mgm. per kilo.	430.3 mgm. per kilo.
Phosphorus extracted with dilute acid	387.4 mgm. per kilo.	290.4 mgm. per kilo.

Dog 209. Bull-terrier, male, weight 13.50 kilos. The parathyroids were removed at 10.30 a.m., March 1, 1912. A slight tremor was noticed at 2.30 p.m., the following day. This became a little more marked during the afternoon but was not severe at 2.30 a.m., March 3. There was pronounced twitching at 10.20 a.m. and the dog was bled to death.

Dog 210. Bull-terrier, male, weight 13.00 kilos. Exsanguinated at 9 a.m., March 8.

TABLE VI.

Analyses of blood from a parathyroidectomized dog and a normal dog.

	PARATHYROIDECTOMIZED	NORMAL
Total nitrogen	3.02 per cent.	2.97 per cent.
Total phosphorus	482 mgm. per kilo.	409 mgm. per kilo.
Phosphorus extracted with acetone	145 mgm. per kilo.	135 mgm. per kilo.
Phosphorus extracted with alcohol and ether	11.7 mgm. per kilo.	12.2 mgm. per kilo.
Phosphorus extracted with picric-acetic acid solution	284 mgm. per kilo.	239 mgm. per kilo.

TABLE VII.

Analyses of serum.

	PARATHYROIDECTOMIZED DOG 212	NORMAL DOG 213
Total nitrogen	9.833 gm. per kilo.	9.350 gm. per kilo.
Total phosphorus	209 mgm. per kilo.	179 mgm. per kilo.
Phosphorus extracted with acetone	129 mgm. per kilo.	133 mgm. per kilo.
Phosphorus extracted with alcohol and ether	Trace	Trace

Dog 212. Bull-terrier, bitch, weight 13.50 kilos. The parathyroids were removed at 11.30 a.m., June 17, 1912. At 4 p.m., the following day, the dog was in tetany and was exsanguinated.

Dog 213. Mongrel, bitch, weight 11.60 kilos. Bled at 4 p.m., June 22, 1912.

TABLE VIII.

Phosphorus in blood from a thyro-parathyroidectomized dog (214) and a normal dog (214B).

	MILLIGRAMS PHOSPHORUS PER KILO OF BLOOD	
	214	214B
Total	436	370
Extracted with acetone	162	140
Extracted with alcohol and ether	8	7
Extracted with picric-hydrochloric acid	233	192
Not accounted for (protein phosphorus?)	33	31

Dog 214. Mongrel, male, weight 12.40 kilos. Parathyroidectomy was performed July 16, 1912. Tremor did not appear, nor was the excretion of phosphorus diminished. At 4.30 p.m., July 20, both thyroids were removed. At 11.30 p.m., the following day, there was slight, occasional twitching. This was more evident at 9 a.m. the next morning and the dog was bled to death.

Dog 214B. Mongrel, male, weight 13.50 kilos. Bled at 9 a.m., August 9, 1912.

TABLE IX.

Phosphorus in serum from parathyroidectomized and normal dogs.

NUMBER	PHOSPHORUS PER KILO OF SERUM			
	Total	Acetone extract	Acid extract	Residue
	mgm.	*mgm.*	*mgm.*	*mgm.*
Parathyroidectomized				
215	212	149	62.3	
217	222	128	76.7	
219*	291	194	87.7	3.8
Normal				
216	157	131	26.9	
218	244	186	44.4	2.4
220	167	110	45.5	

*Complete thyroidectomy.

Dog 215. St. Bernard, male, weight 26.0 kilos. The parathyroids were removed in the afternoon of October 31, 1912. Slight tremor appeared at 6 p.m., November 3. At 8.30, twitching was quite pronounced and the dog was bled.

Dog 216. Mongrel, male, weight 14.0 kilos. Bled at 8 p.m., November 16, 1912.

Dog 217. Bull-terrier, male, weight 11.0 kilos. Parathyroidectomy was performed on the morning of November 25, 1912. Tremor was doubtful at 9 a.m., November 27 but, at 3 p.m., the dog had a severe attack of tetany and at 3.45 was exsanguinated.

Dog 218. Mongrel, male, weight 9.45 kilos. Bled at 4.30 p.m., February 12, 1913.

Dog 219. Mongrel, male, weight 14.7 kilos. Four parathyroids were removed on December 19, 1912. As no symptoms appeared, the right thyroid was removed on December 22. This was also without apparent effect and at 3 p.m., December 25, the other thyroid was removed. At 8 p.m., December 29, there was slight, though unmistakable, twitching. No change was observed at 10.30 p.m. and the dog was bled. A severe attack of tetany occurred during the bleeding.

Dog 220. Mongrel, male, weight 16.0 kilos. Bled at 10.30 p.m., January 12, 1913.

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FURTHER METABOLISM EXPERIMENTS UPON PARATHYROIDECTOMIZED DOGS.

By ISIDOR GREENWALD.

(From the Laboratories of Pathology and of Biological Chemistry of Columbia University and the Chemical Laboratory of the Montefiore Home.)

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In a previous paper,[1] the author described a series of metabolism experiments upon parathyroidectomized dogs. The most striking change in the metabolism observed after removal of the parathyroids was the very marked diminution in the excretion of phosphorus in the urine. This was not accompanied by an increase in the elimination of phosphorus in the feces. Apparently there was a retention of phosphorus in the body.

One of the questions that then arose was: Is this retention of phosphorus a primary metabolic disturbance or is it secondary to a retention of sodium or of potassium or of both? The experiments here reported were designed to answer this question. The results leave no doubt that the retention of phosphorus is primary. In none of the experiments was there any indication of a retention of sodium or of potassium preceding that of phosphorus. In three, the fall in the phosphorus elimination occurred a day before the excretion of sodium and potassium was affected. In the other two, the excretion of all three substances was diminished on the day of the operation.

The significance of these experiments is not altogether clear. It may be that, after parathyroidectomy, the ratio between the concentrations of calcium and of sodium and potassium in the body-fluids is altered not so much by loss of calcium as by retention of sodium and potassium. A few analyses of serum indicate that this is the case. Whether or not such a change can be regarded

[1] Greenwald: *Amer. Journ. of Physiol.*, xxviii, p. 103, 1911.

as the cause of tetany is, of course, questionable. However, since these changes in the excretion of phosphorus, sodium and potassium are so marked, it seemed desirable that the content of these substances in the tissues be investigated. Some work has already been done on the amount and nature of the phosphorus compounds of the blood and serum of normal and parathyroidectomized dogs. An account of these experiments is given in the following paper.

As in the previous experiments, the author is indebted to Dr. W. G. MacCallum, who was kind enough to operate upon the animals required.

EXPERIMENTAL.

The conduct of the experiments was similar to those previously reported.[2] The analytical procedures were the same with the following additions and changes:

Phosphates. Titration with uranium acetate, using cochineal as indicator.

Mineral acidity. The Folin method.[3]

Potassium and sodium. Fifty cc. of urine are ashed, with the aid of a little sulphuric acid, in a platinum dish. The residue is dissolved in water and treated with an excess of barium hydroxide. The precipitate is filtered out and washed thoroughly. A slight excess of sulphuric acid is added to the filtrate. After filtering from the barium sulphate, the liquid is evaporated to dryness in a weighed platinum dish, which is then ignited and weighed. (The trace of calcium present is included but may be disregarded.) The residue is dissolved in about 20 cc. of water, 2 cc. of glacial acetic acid are added and the potassium precipitated as the cobaltinitrite by adding 20 cc. of the solution of Adie and Wood,[4] drop by drop (this was found to be necessary to ensure correct results). After standing over night the precipitate is filtered off on a Gooch crucible fitted with a piece of filter paper, and washed with 10 per cent acetic acid, as recommended by Thompson and Morgan,[5] whose directions are followed from this point. The crucible with the precipitate is placed in the beaker in which the precipitation has been made, an excess of barium hydroxide solution is added and the liquid is boiled for two or three minutes. The precipitate of cobalt hydroxide is filtered off and washed. The filtrate and washings are diluted to a definite volume, generally 250 cc. This liquid is then run into a hot, dilute standard

[2] Greenwald: *loc. cit.*

[3] Folin: *Amer. Journ. of Physiol.*, ix, p. 265, 1903.

[4] Adie and Wood: *Journ. of the Chem. Soc.*, lxxvii, p. 1076, 1900.

[5] Thompson and Morgan: *Journ. of Ind. and Eng. Chem.*, iii, p. 398, 1911.

solution of potassium permanganate, containing sulphuric acid, until this is decolorized. From the volume required, the amount of potassium is calculated and from this and the weight of the mixed sulphates, the amount of sodium.

TABLE I.

Excretion of phosphorus after incomplete parathyroidectomy. Dog 205.

DATE 1912	WEIGHT	VOLUME	SP. GR.	PHOSPHATES	REMARKS
February	*kilos*	*cc.*		*mgm.* P.	
6	11.67	385		361	
7	11.72	360	1.019	366	
8	11.70	380	1 020	393	
9	11.70	400	1 019	406	
10	11.70	450	1 018	511	
11	11.70	400	1 020	438	
12		415	1 021	467	Parathyroidectomy,
13	11.70	400	1 017	353	12 noon, Feb. 11.
14	11.72	330	1 022	324	
15	11.72	380	1 020	445	

At no time did the dog show any evidence of a parathyroid insufficiency. The excretion of phosphorus remained almost unchanged.

TABLE II.

Urinary data. Dog 209.

DATE 1912	VOLUME	SP. GR.	NITROGEN	CHLORINE	PHOSPHATES	POTASSIUM	SODIUM
February	*cc.*		*grams*	*mgm.*	*mgm.* P.	*mgm.*	*mgm.*
27	600	1.016	7.567	605	651	1027	370
28	460	1.020	7.741	240	562	727	32
29	400	1.018	6 760	173	493	697	28
March							
1	430	1.017	6 578	163	437	627	54
2	200	1.031	5.675	29	11	618	8
3	420	1.014	5 858	102	14	278	28

The dog weighed 13.50 kilos. Parathyroidectomy was performed at 10.30 a.m., March 1. A slight tremor was observed at 2.30 p.m., March 2. This gradually increased in severity until 10.20 a.m., March 3, when the dog was exsanguinated. In spite of the very marked diminution in the excretion of phosphorus on the day of the operation, the elimination of potassium remained unchanged until the following day.

TABLE III.

Urinary data. Dog 212.

DATE 1912	WEIGHT	VOLUME	SP. GR.	NITROGEN	PHOSPHATES	MINERAL ACIDITY
June	*kilos*	*cc.*		*grams*	*mgm. P.*	*cc. $\frac{N}{10}$ acid*
13	13.55	540	1.026	12.65	746	440
14	13 50	550	1 022	11.38	718	624
15	13.50	490	1.021	9.56	616	595
16	13.50	400	1.024	9.51	639	619
17	13.60	460	1.026	9.40	551	561
18		140*	1.036	4.80	115	127
18 p.m.		360	1.021	8.34	148	317

* A small quantity of urine was lost.

The parathyroids were removed at 11.30 a.m., June 17. Symptoms appeared on the afternoon of the following day. The dog was bled to death at 4 p.m. On the day of the operation the lessened phosphorus excretion was paralleled by diminished mineral acidity. The latter was increased somewhat on the following day, due, presumably, to a retention of potassium and sodium.

TABLE IV.

Urinary data. Dog 214.

DATE 1912	VOLUME	SP. GR.	NITROGEN	PHOSPHATES	CHLORINE	MINERAL ACIDITY
July	*cc.*		*grams*	*mgm. P.*	*mgm.*	*cc. $\frac{N}{10}$ acid*
9	300	1020	6.566	369		
10	360	1020	8.335	511		
11	320	1020	8.170	476		
12			4.960	317		
	430	1026				
13			4.960	317		
14	550	1013	8.880	531	256	435
15	430	1015	7.163	454	241	365
16	350	1020	7.152	425	210	333
17	300	1028	7.290	435	471	96
18	400	1016	8.145	317	162	359
19	350	1021	8.046	348	249	390
20	230	1027	10 000	481	273	453
21	560		8.108	257	245	221
22	450	1016	8.106	147	147	251

The dog weighed 12.40 kilos. Parathyroidectomy was performed at 11 a.m., July 16. There was a slight fall in the excretion of phosphorus but no other indication of parathyroid insufficiency. On July 20, at 4.30 p.m., both thyroids were removed. The output of phosphorus immediately decreased. Slight twitching was noted at 11.30 p.m., July 21. The next

morning, the twitching was more marked and the dog was killed. The very marked drop in the mineral acidity on the day of the first operation cannot, at present, be satisfactorily explained. After the second operation, the mineral acidity fell with the excretion of phosphorus.

TABLE V.

Urinary data. Dog 215.

DATE 1912	NITROGEN	PHOSPHATES	CHLORINE	POTASSIUM	SODIUM
October	*grams*	*mgm.* P.	*mgm.*	*mgm.*	*mgm.*
26	10.73	874	800	823	166
27	8.39	717	533	629	105
28	12.48	1035	924	1282	466
29	8.89	744	382	694	140
30	12.68	1166	1560	1275	519
31	8.38	685	637	619	383
November					
1	10.36	560	1668	869	1143
2	5.08	171	312	166	80
3	14.63	832	1184	791	125
3 p.m.	7.17	475	629	685	75

The dog weighed 26.0 kilos. Parathyroidectomy was performed in the afternoon of October 31. Slight tremor was observed at 6 p.m., November 3. At 8.30 p.m., there was severe twitching and, at 9 p.m., the dog was killed. On the day of the operation there was a pronounced fall in the elimination of phosphorus. The usual amount of potassium was excreted and the output of sodium was greater than on any other day.

TABLE VI.

Urinary data. Dog 217.

DATE 1912	NITROGEN	PHOSPHATES	POTASSIUM	SODIUM
November	*grams*	*mgm.* P.	*mgm.*	*mgm.*
21	5.479	393	549	291
22	6.340	494	599	268
23	6.803	520	620	277
24	9.202	731	1133	702
25	6.571	533	757	218
26	7.352	242	320	53
27	6.750	310	235	21
27 p.m.	2.623	113	212	18

The dog weighed 11.0 kilos. The parathyroids were removed on the morning of November 25. At 3 p.m. November 27, the dog was in tetany and was exsanguinated. In this experiment the excretion of potassium and sodium paralleled that of phosphorus.

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EXPERIMENTAL STUDIES ON THE NERVOUS MECHANISM IN THE PRODUCTION OF HYPERPLASIA

Walter Timme, M.D.

MEMBER OF THE NEW YORK NEUROLOGICAL SOCIETY; ASSISTANT PHYSICIAN, NEUROLOGICAL INSTITUTE, NEW YORK CITY

Du Bois-Reymond in 1843 (1) first gave us our definite knowledge of the electrical properties of living tissue. A muscle with its nerve, removed from the body, gives different electrical potentials at the cut surface and at the longitudinal uninjured one. Therefore, a flow of current, measurable with a galvanometer, is determined between the two points. This makes of such a muscle-nerve preparation an actual electric battery. Du Bois-Reymond maintained that these currents existed continuously in the living body during life. Claude Bernard (2), opposing him, stated that the current is found only in active tissue and not in quiescent muscle; and Hermann (3), following him, found that in uninjured muscle the potentials at various points were equal, and therefore no current was produced. He called the "natural currents" of Du Bois-Reymond, "currents of injury." Both of these objections, however, seem invalid; for there is no such thing in the living body as a quiescent muscle, and one cannot determine differences of potential by taking points on the surface of the muscle only. In fact, the chemical changes constantly taking place coincident with metabolism in all tissue must determine a current flow. I do not desire at this time to present any arguments either for or against the theory of a constant flow of current pervading all the living structure, produced by these living processes of metabolism; and conveyed by the various nerve elements to the controlling and regulating central systems, thence in its modified form returning to its points of original departure, completing the cycle. Sufficient to state now, that there is a great probability of the presence of such a circulating nerve current; circulating, for it has never seemed reasonable to suppose that a nerve impulse, *if electrical in*

character, could be initiated at one end of a path to produce an effect at the other end, without there being a completed circuit back to the point of original departure. The existence of complete circuits for the nerve impulses to travel might necessitate the presence of many more axones than nerve cells. Of course, this would be in opposition to the present theory of neurones each with its *one* axis cylinder. But both Nissl (4) and Bethe (5) state that from their studies they are convinced of the far greater number of axones present in the cerebrum than nerve cells, and advance this fact as destructive of the neurone theory of Waldeyer. Furthermore, Ramon y Cajal (6), in describing certain end-organs of the skin, states that "each nerve trunk divides into *two* branches which surround more or less perfectly the hair follicle, each with a free extremity. The ordinary end organ is innervated by *two* independent systems of fibers." So that we see that histologically there may be a basis for a completed circuit for the nerve impulse.

Proceeding then upon the above supposition, that the nerve impulses produced by metabolism travel in certain circuits, it remains to find an organ with its nervous mechanism suitable for experimentation. For many reasons, the vagus nerve (below the branches given off to the esophageal plexus), and its related organ, the stomach, offer ideal conditions. This organ receives fibers from both the vagus and the sympathetic; and moreover, these fibers do not travel in a common trunk, as is the customary condition elsewhere in the body, but arrive independently; uniting only at the plexuses of Meissner and Auerbach within the stomach wall. L. R. Müller (7) is authority for the statement that the vagus is differentiated from all other cranial nerves, for the reason that it serves the abdominal organs without the interposition of sympathetic ganglia; and that there is a distinct antagonism between the vagus fibers and the sympathetic to the stomach, both physiological and pharmacological. And Haidenhain (8) states that the physiological functions of these nerves on the gland cells of the gastric mucosa are respectively "secretory" and "trophic."

Now, if, without disturbing too much the motility and function of the stomach, we can succeed in causing the nerve current produced by the activity of the gastric cells to flow in diminished volume via the vagus, the excessive remainder must travel by the

sympathetic. If it travels this route it incidentally stimulates the control exerted by this system over the *trophic* functions of the gastric cells, i. e., their growth, reproduction and repair. Our problem was to determine the truth or falsity of this inference. Furthermore, the method of operating upon the vagi, heretofore, was simply to divide them. (Schiff (9), Pawlow (10), Cannon (11), v. Yzeren (12)). This not only actually interfered with their part entirely in the digestive process, but also provided a situation rarely, if ever, met with in actual life—a distinct nerve separation. By the present method of vagus ligation, while the nerves are not left entirely intact, yet a sufficient number of fibers are left to carry on some of the vagus function, and a condition frequently met with in actual life is reproduced—namely a pressure neuritis.

Operation.—The animals used for experimentation were cats, and they were prepared for operation by the usual methods. The anesthetic was ether administered by the insufflation method of Meltzer and Auer. As there was much difficulty experienced in passing a catheter through the larynx, tracheotomy was resorted to as a preliminary in each case, and the catheter then passed directly into the trachea. Air was given with the ether in considerable quantity, and every few minutes the administration was stopped to allow the animal to breathe normally. Then the thorax was opened by an incision parallel to the ribs at the seventh interspace, left side, a self-retaining retractor inserted, and its blades separated widely. This exposed the thoracic viscera and gave ample freedom for the purpose in view. The lungs and heart were then pushed upward, and gauze inserted to keep them from the field. After cutting through the posterior layers of pleuræ, the esophagus was easily within reach from a point two inches above the diaphragm to the diaphragm, and the anterior vagus (the left) was then exposed. It was ligated with a coarse though soft cord, and rather less force exerted in tying than is used to tie off a medium-sized artery. The same process was undergone with the posterior vagus (right), the thoracic wound closed, catheter removed and trachea sutured, finally closing the neck wound. The thorax was tightly bandaged, the neck wound dressed, and the animal was left to recover.

Post-operative.—For a few days, the cats would take only milk and seemed under much shock, but gradually other food

would be taken; so that by the end of a week or ten days they would eat meat and fish also. Of nine animals thus operated upon, one died of inanition after seven days, two directly after operation, and one of sepsis due to scratching off the neck bandage. The other five lived. In cats No. 1 and No. 2, only the left vagus was tied off; in the others, both vagi were ligated. All five animals gained weight, but acted rather sluggishly. They were killed at various times between 111 and 140 days after operation. In each case the animal was killed between five and six hours after a full meal. As controls, three well nourished animals were used. These were fed similarly to those operated upon and also killed 5–6 hours after a full meal. These are designated in the table as N1, N2, and N3.

Upon opening the abdomen in the operated animals, the most noticeable condition was the still full appearance of the stomach, and the hardness of the stomach walls. The large intestine also seemed unduly large in calibre and in length. In the thorax, below the ligature, the vagus seemed like a silver thread, about one quarter to one third the size of the portion above the ligature. Upon opening the stomach from cardia to pylorus it was found filled to its capacity with only slightly digested masses of meat, almost dry, and the various masses still discrete. In the normal cats, digestion had progressed until the mass was gelatinous, and the various particles thoroughly coated with gastric juice; and the walls were neither much distended nor hard. The next point of difference between the operated animals and the controls was in the thickness of the stomach wall. In the operated animals, this varied between two and three times the thickness of the normal one; and when the contents were removed, and the stomach collapsed, bands of muscular tissue showed plainly in its walls. The stomach felt tough and very thick and nodulated between the fingers, while in the controls, the walls were softer and more delicate and the fingers could appreciate objects through the thickness of the wall. The most marked change in gross appearance, however, was seen in the rugæ. As seen by the photographs, cats No. 1 and No. 3 have these rugæ standing out prominently in all directions—a very redundant mucous membrane, feeling much like a mass of worms, while in the control cat N1, they were longitudinal and orderly in arrangement, and easily smoothed out under slight pressure. As will be seen by

the table, the colon also was larger and of greater calibre in all the cats operated upon than in any of the controls. These results agree in a measure with those of Schiff (9), who divided the vagi

All three photographs represent the everted stomachs of animals, No. 1, N1, No. 3. From the table, we see that cat No. 1 suffered ligation of only the left vagus, cat No. 3 of both vagi, and cat N1 was the control. The stomach was cut from the cardia to the pylorus, and simply everted and photographed (all three at the same exposure). The great redundancy of the mucous membrane in the operated animals is at once evident; there is even seen the excess of the rugæ in cat No. 3 over cat No. 1, and the disorderly arrangement of the rugæ.

below the diaphragm. His animals thrived and fattened; from which however he drew the erroneous conclusions that no secretory nerves pass via the vagi to the stomach; in contradiction to which Pawlow (10) shows conclusively that stomach glands are activated by impulses from the vagi. The largely undigested condition of the stomach contents in our operated animals agrees with Pawlow's contention. Cannon (11) also states that in six hours a normal cat's stomach is usually empty but is delayed by division of vagi; which view agrees with our findings in vagus ligation. W. von Yzeren (12) states that after vagotomy the stomachs are hard and the pressure of the walls slightly increased—also in agreement with the present results. But none of these authors makes special mention of any increase in the size or thickness of the various coats of the stomach, especially of the increase of the glandular one. It is our belief that that marks the distinction between the results of vagotomy and a vagus pressure neuritis, such as is produced by this method.

Microscopically, the appearance of the stomach wall was distinguished from the normal, by the great excess of submucous areolar tissue, by the great bands of muscle fibers, both longitudinal and transverse, and by a marked increase of the convolutions of the mucous membrane and the number of the stomach glands. To properly estimate this latter increase, the

Cat No.	1	2	3	4	N1	N2	N3
Operation	Left vagus tied off		Both vagi tied off		——	——	—
Days killed after operation	111	151	109	100	——	——	——
Stomach wall	thick, large, hard, and resistant to pressure; nodulated				soft, thin, pliable, smooth and uniform		
Mucous membrane	redundant; many rugæ irregular; (photograph)		still more redundant; (photograph)		rugæ orderly and parallel; not redundant; (photograph)		
Pressure to flatten rugæ	——	1,560 mm. water	——	1,480 mm. water	——	660 mm. water	723 mm. water
Colon, length	312 mm.	317 mm.	324 mm.	——	195 mm.	207 mm.	——
Colon, circumfer.	60 mm.	69 mm.	68 mm.	——	32 mm.	41 mm.	——

following method was adopted—which was kindly suggested by Dr. W. G. MacCallum. Two stomachs, one of a control animal, were distended with 5 per cent. formalin solution, directly after killing the animals, at a pressure sufficient to flatten out the rugæ. At this pressure, which was recorded, the cardiac and pyloric ends were ligated and after being measured, the whole mass placed in a jar of 5 per cent. formalin. After a few days, the distention becoming permanent, small portions of various parts of both stomachs were cut out, and horizontal sections through the glands parallel to the stomach surface were made and stained with hematoxylin. These transverse sections of the gastric glands allowed of a comparatively easy count of the number of glands. Even without actual count, the number per field in the operated animal could easily be seen as increased over that in the control. The actual increase was estimated with the result as given in the table.

The pressures necessary to flatten out the rugæ were in the normal cat, N2, 660 mm. water and in operated No. 2 animal, 1,560 mm. The size of the stomachs when thus distended was for the normal animal, N2:

Greater curvature ..... 250 mm.
Lesser curvature ..... 85 mm.
Circumference ..... 235 mm.

In the operated animal No. 2,

Greater curvature ..... 291 mm.
Lesser curvature ..... 83 mm.
Circumference ..... 242 mm.

In the control animal N2,

Lenth of colon ..... 207 mm.
Circumference ..... 41 mm.

In the operated animal No. 2,

Length of colon ..... 317 mm.
Circumference ..... 69 mm.

The glands in the transverse sections were counted in six separate fields and gave the following results (per sq. mm.):

Control N2.	Operated Animal No. 2.
353	420
299	466
284	501
300	393
296	408
302	440
1,834	2,628

giving an average of:

305 + per sq. mm. 438 per sq. mm.

The approximate estimates of the superficial areas of these stomachs are 19,539 sq. mm. and 22,627 sq. mm. respectively. This will give a total number of glands on the stomachs of cat N2 of 5,978,934 and of cat No. 2 of 9,910,626—a marked increase. The stroma between the glands was much diminished in the cat No. 2 as compared with the control animal, and the glands therefore placed more closely. The number of cells in each gland of both controls and operated animals was about the same. Other changes microscopically observed are not of interest in the present paper and so will not be recorded here.

In the colon there was produced both an increase in length and in calibre, with a corresponding increase in the number of follicles of Lieberkuehn. In this connection, we see a correspondence with the conditions presented in Hirschsprung's

disease (giant colon) for which many causes have heretofore been ascribed; thus (1) congenital, (Hirschsprung, Concetti, Duval et al.); (2) congenital dilatation plus mechanical and inflammatory changes and hypertrophy (Genersich); (3) stoppage and decomposition of intestinal contents (Marfan, Griffith, Frommer); (4) valves, folds and crevices causing stagnation, dilatation and hypertrophy (Perthes, Marchand); (5) volvulus of the flexure (Frommer, Barth); (6) spasm of sphincter ani (Fenwick, Wilms, Bertelsman).

To produce the dilatation of the colon without producing an effect upon the stomach, it is possible that some of the vagus filaments below the stomach are compressed without a corresponding pressure on the respective sympathetic fibers. It seems likely therefore that in the pathogenesis of Hirschsprung's disease, a pressure neuritis on the terminal vagi fibers may be found to play an important rôle.

We then see that a pressure, long continued, upon the vagus below the esophageal plexus, will produce in the stomach an actual cell increase in the mucous membrane of that organ. This increase of the number of cells is, however, not coincident with an increase in their functional activity as we have seen, but in a diminished activity or perverted function. In other words, the trophic control over the gastric cells,—that having to do with their growth and reproduction,—was excessively stimulated; the control of their functional activity and that of the stomach as a whole,—the production of the gastric juice, the expulsion of the contents from stomach to intestine—was greatly in abeyance.

The supposition, therefore, of the nerve current passing in the circuit of the sympathetic,—the solar plexus and the splanchnics—in increased quantity as a result of the partial closure of the vagus path, and causing increased activity in those centers, with diminished vagus activity, is supported by the results obtained in these experiments. (Do these results perhaps also explain so-called vagus inhibition?)

Can we deduce possibly other far-reaching effects from our results? Can we say that a long-continued pressure upon some one mixed nerve, containing both sympathetic and central fibers, if acting unequally upon these fibers, producing greater pressure effects upon the cerebrospinal ones than upon the sympathetic;—can we say that such a pressure will produce on the cells of

the tissue to which the nerve trunk is accredited an activity resulting in their numerical increase producing a true hyperplasia with a diminished or perverted function? If so, have we not in such a pressure one of the factors in the production of neoplasms?

In conclusion, I beg to acknowledge my indebtedness for many valuable suggestions, and for actual assistance in my experiments, to Dr. W. G. MacCallum, who also kindly allowed me the use of the Pathological Laboratory at the College of Physicians and Surgeons, and to whom I herewith tender my thanks and appreciation; also to Dr. Pearce Bailey, of the Neurological Institute, whose stimulating criticisms aroused the necessary energy to prosecute the work; and finally to Miss Laura Stryker, of the Pathological Department of Columbia University, who kindly undertook to prepare the microscopic material for me.

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PREFACE.

The papers included in this volume represent the results of the work done in the Department of Pathology of the College of Physicians and Surgeons during the Collegiate years 1914-16.

The cost of reprinting and issuing the studies is defrayed by a special grant from Columbia University and not as heretofore by the Alumni Association of the College of Physicians and Surgeons.

W. G. MacCallum, M.D.,
Director.

Department of Pathology
College of Physicians and Surgeons
Columbia University, New York
December, 1916

1. *Three Cases of Adams Stokes Syndrome with Histological Findings.* Adele Oppenheimer, B. S. Oppenheimer.
2. *Mechanism of Anaphylatoxin Formation.* J. W. Jobling and W. Petersen.
3. *Removal of Calcium from the Blood by Dialysis in the Study of Tetany.* W. G. MacCallum, R. A. Lambert and Karl M. Vogel.
4. *The Role of the Spleen in Blood Formation.* Dudley H. Morris.
5. *Relation of Bacteriolysis to Proteolysis.* J. W. Jobling and W. Petersen.
6. *Further Experiments Upon the Effects of Extirpation of the Thymus in Rats, with Special Reference to the Alleged Production of Rachitic Lesions* Alwin M. Pappenheimer.
7. *Bacterial Antiferments.* Studies on Ferment Action. J. W. Jobling and W. Petersen.
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9. *Zur Biologischen Bedeutung der Ungesättigten Fettsäuren.* J. W. Jobling and W. Petersen.
10. *Malignant Melanotic Tumor Primary in the Adrenals.* A. M. Pappenheimer.
11. *Effect of Penetrating Rays Upon Tuberculosis.* Leo Kessel and M. J. Sittenfield.
12. *The Therapuetic Action of Iodin.* J. W. Jobling and W. Petersen.
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14. *Cholesterin Metabolism of Hens Egg During Incubation.* J. Howard Mueller.
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16. *Aortic Aneurysm Perforating Superior Vena Cava.* W. G. MacCallum.
17. *New Ectoparasitic Trematodes.* G. A. MacCallum.

18. *Assimilation of Cholesterol and Its Esters.* J. Howard Mueller.

19. *Eosinophilic Infiltration of Pancreas.* H. A. Riley.

20. *Experimental Study of Blood Glycolysis.* G. M. MacKenzie.

21. *Ligation of Splenic Vessels as Substitution for Splenectomy.* A. Troell.

22. *Amyloidosis.* R. A. Lambert.

23. *Report on Two Forms of Cardiac Abnormality.* H. A. Riley.

24. *Some Attempts to Produce Experimentally Conditions of Sympatheticotonus, Vagotonus and Hyperthyroidism.* A. Troell.

25. *Some Reactions of Blood Vessels to Certain Chemicals.* Dr. I. Adler.

26. *Tissue Cultures in the Investigation of Cancer.* R. A. Lambert.

27. *A Comparison of the Results Obtained by the Colorimetric and Gravimetric Determinations of Cholesterol.* J. Howard Mueller.

28. *Limitations to the Cultivation of Mammalian Tissue in Vitro.* R. A. Lambert.

29. *The Cytology of the Exudate in the Early Stages of Experimental Pneumonia.* Frank A. Evans.

30. *Some New Species of Parasitic Trematodes of Marine Fishes.* G. A. MacCallum.

31. *A Study of the Involutional Changes Which Occur in the Adrenal Cortex During Infancy.* R. W. Lewis and A. M. Pappenheimer.

32. *Technique of Cultivating Human Tissues in Vitro.* R. A. Lambert.

33. *The Influence of Autolysis Upon Cholesterol Esters.* J. Howard Mueller.

34. *Lipoids as Inhibitors of Anaphylactic Shock.* Studies on Ferment Action. James W. Jobling and William Petersen.

THREE CASES OF ADAMS-STOKES SYNDROME WITH HISTOLOGICAL FINDINGS *

ADELE OPPENHEIMER, A.M., AND B. S. OPPENHEIMER, M.D.
NEW YORK

Although it is established that a complete transverse lesion of the stem of the auriculoventricular conducting system or of its two main branches results in auriculoventricular dissociation, and although a few years ago the problem of heart-block seemed almost settled by the discovery of lesions in the conducting system, it is now apparent that the solution of this problem is not so simple as it appeared at that time. There are cases on record in which heart-block was proved beyond a doubt, but still no lesions in the hearts adequately explaining the failure of the conduction of impulses from auricle to ventricle were to be found.†

The three cases to be presented show three very different histological conditions in the conducting system: and it is somewhat unfortunate that the clinical data do not give us such definite information of the mechanism of the heart as can be positively correlated with these post-mortem findings.

Three hearts from patients with a clinical history suggesting heart-block were kindly entrusted to us for microscopic examination; two by Dr. MacCallum and one by Dr. Bishop.

HEART 1.—*Case Report.*—The case report has been given by Dr. Bishop.[1] The patient, a watchman, aged 75, was admitted to Lincoln Hospital, Oct. 22, 1908. His family history was negative. He had had measles, whooping-cough and small-pox when a child. Later in life he had had muscular rheumatism and a sunstroke. He had drunk whisky moderately. Syphilitic history was denied. The patient was described as having a slow pulse, vertiginous and syncopal attacks and rapid venous pulse. The radial artery was not thickened, even to the degree that would be expected in a patient of his age. A polygraphic tracing was published in the original article.

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* From the Pathological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York City; read in abstract before the New York Pathological Society, Nov. 12, 1913.

† A. F. Stanley Kent has described in detail another connection between the auricle and ventricle beside the bundle of His, situate at the right lateral border of the human heart. (Proc. Roy. Soc., London, 1914, Series B., lxxxvii, No. B594, Biological Sciences, p. 198.)

1. Bishop: Am. Jour. Med. Sc., 1910, cxxxix, 62.

Macroscopic Examination of the Heart.—The post-mortem examination of the heart was made by Dr. Larkin.[2] "The heart showed a calcareous nodule, situated precisely in the path of the bundle of His. This nodule was evidently the cause of the complete heart-block observed during life."

The heart was received from Dr. Bishop in formaldehyd solution and the measurements of the preserved heart revealed nothing of interest.

The edges of both mitral cusps were shrunken and deformed and one could feel a hard calcareous deposit all along the attachment of the posterior cusp. The aortic cusp of the mitral valve was thickened throughout and contained irregular, rough, hard calcareous deposits. This calcareous mass was continuous with that described above in the upper portion of the interventricular septum and in the space between the anterior and right posterior cusps of the aortic valve, that is, directly in the normal pathway of the bundle of His. The cusps of the aortic valve were markedly thickened. The wall of the aorta itself showed patches of atheroma. The coronary arteries, both right and left, presented a very marked sclerosis with patches of atheroma.

Microscopic Examination.—Such a portion of the heart was removed for microscopic examination as would include the calcareous mass which seemed macroscopically to be situated precisely in the path of the bundle of His; namely, such a portion of the septum between the two halves of the heart as included the node, stem, and the branching of the auriculoventricular system. The upper margin of this block ran along the upper edges of the aortic leaflets, and the lower margin parallel with this 3 cm. below the lowest part of the pars membranacea septi.

This piece of tissue was decalcified with nitric acid, embedded in paraffin, cut from above down, parallel with the upper margin, in sections 15 to 19 microns thick. Altogether 2520 sections were cut and for a preliminary study every fortieth mounted. Finally about every fourth section from Section 1322 (where the node was first seen) to 1618 (well beyond the break in the continuity of the auriculoventricular system), and about every tenth from 1618 to 2440, was mounted. All the sections were stained with Weigert's iron hematoxylin and van Gieson's picric acid fuchsin solution except Sections 1392, which was stained with hematoxylin and eosin, and 1334, which was stained by Roehl's method for calcium.

The auriculonodal connections are attenuated (especially the strand to the left) but are uninterrupted. A very few round cells and still fewer polymorphonuclear cells are found at this junction and in the node itself.

The node lies, as usual, to the right of the central fibrous body, but is peculiar in that it is largely cut off from the auricular musculature by fatty tissue. It consists of a network of pale, fine muscle-fibers with crowded nuclei. The nodal artery shows increase of connective tissue in the intima and media; indeed there is sclerosis of the vessels throughout the whole of all the sections.

The node and stem are continuous. The stem is characterized by a greater vascularity, a more marked cellular infiltration and by the presence of more connective tissue than the node, which characters are so marked in the auriculoventricular system at and near the bifurcation of the stem as undoubtedly to be beyond normal limits. The stem progresses normally through the membranous septum in a short course and comes to lie under the endocardium of the left ventricle, when abruptly at its forward end it abuts on a calcified necrotic sphere there bifurcating to form what were judged to be stumps of the right and left

2. Bishop and Larkin: Proc. New York Path. Soc., 1909, New Series IX, 58.

branches. The calcified sphere, which lies directly across the path of the stem at its bifurcation, stretches from the endocardium of the left ventricle almost to that of the right.

This calcified sphere forms part of an area of calcification which begins just below the anterior aortic (coronary) cusp and involves the stump of the mitral valve, the connective tissue of the auriculoventricular ring and the undefended space, and the surrounding musculature, namely, that of the left auricle and of the basal part of the interventricular septum and of the auriculoventricular bundle. The area consists in the main of nodules of calcified necrotic material well encapsulated by connective tissue sheaths. In and immediately about some of the encapsulated nodules there is increased vascularity accompanied by pus cells (mostly round cells but not a few polymorphonuclears). There is also here and there the typical invasion of the calcific nodules by young connective tissue with an occasional giant cell.

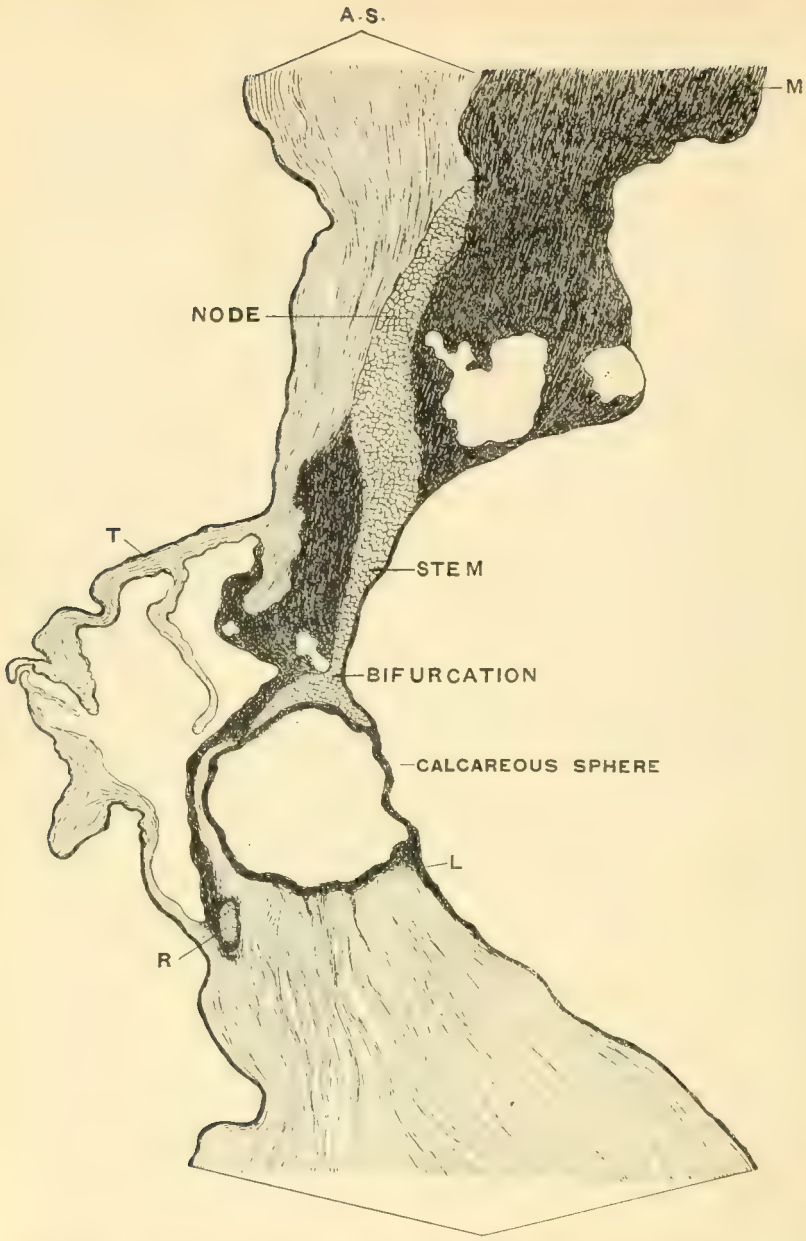
At one level, one of these calcific nodules appears to the right of the ventricular end of the stem abutting on its narrowest part, and comes to lie in an indentation of the stem giving the appearance of compressing it. This occurs not far from the bifurcation, namely, at the forward end of the stem, which is composed mostly of thin-walled engorged blood-vessels and connective tissue and proportionately very few muscle-fibers. The undoubted increase in connective-tissue elements and vascularization which characterizes the ventricular portion of the stem also exists in the stumps of the right and left branches.

The stumps just mentioned are, judging by their position, probably the true branches and not the result of a mere splitting of the stem owing to pathological conditions. The lesion in this angle is, as has already been mentioned, a large calcific necrotic sphere which separates the stem and stumps from the rest of the right and left branches proper (Figure 1). The left branch proper is easily identified, but the identification of the continuation of the right branch we cannot assert with the same conviction.

Even on a naked-eye study of the sections it is clear that as one goes from the aortic valve toward the apex of the heart the calcific, necrotic focus involves a more and more extensive area and compromises the auriculoventricular system more and more. And microscopic study reveals that in Section 1462 there is no viable muscle-fiber in the stump of the right branch and that in Section 1480 there is not even an indication of the stump of the right branch,[3] that in Section 1502 there are no muscle-fibers in the stump of the left branch, and that there is in the region of Section 1544 from above down a hiatus of about 1 mm. (1.026 mm.) in the stem where there is no viable musculature. At this level the stem region consists of vascularized connective tissue with much cellular infiltration (mostly round cells, an occasional polynuclear, and a few cells larger than round cells characterized by more cytoplasm and a less compact nucleus).

To summarize, there are for a short distance (examining from above down) no muscle-fibers in the stem. Above this hiatus a large calcific necrotic sphere intervenes between the stem at its bifurcation on the one hand and the right

3. In spite of the absolute break in the continuity of the right branch, impulses may yet have passed from the stump of the right limb through the ordinary ventricular musculature to the ventricular chamber, for it is not unlikely, judging by the anatomical findings in the hearts which we have studied and by physiological considerations, that the auriculoventricular system is not completely isolated between its two ends, namely, all along the line from the auriculonodal connection to the Purkinje fiber-ventricular junction.



Projection drawing of Sections 1438 and 1442, Case 1.—Showing an old calcareous focus, surrounded by connective tissue, which causes complete interruption of the bundle of His. At the bifurcation of the stem a calcific nodule (larger in the following sections) seems to press in upon it. This indented region of the body of the stem and the stumps of its two limbs present manifestly more thin-walled blood vessels and more connective tissue than is normal. At a lower level the necrosis encroaches on the stem to such an extent that it is entirely lacking in muscle-fibers for about 1 mm. from above down. Fibrous tissue is dark; necrotic material is dotted; *A-V* system is honeycombed; *A-S*, auricular septum; *L*, region where isolated left branch of *A-V* system appears in sections lower down; *M*, mitral valve; *R*, isolated right branch; *T*, tricuspid valve; *V-S*, ventricular septum.

and left branches on the other, and it is only below this hiatus that one branch (namely, the left) is continuous with the body of the stem. In brief, the right and left branches are cut off from the auriculonodal junction because necrotic tissue is situated in the path of the auriculoventricular bundle.

The two branches, cut off as they are from the node, present no abnormalities in their structure.

It should be borne in mind that the identification of the "main" part of the right branch as it descends downward is not absolute, since it is nowhere continuous with the stem, and was judged by its location, the lack of density of its muscle-fibers and its isolation. Farther up, the muscle-fibers of the right limb are slightly finer than those of the surrounding musculature, farther down, slightly coarser with occasionally a clear central space. At one level a small group of muscle-fibers which lies between the right branch and the epicardium, presents great resemblances to the right branch, some of its muscle-fibers have even a clear central space so that this segment of musculature is indistinguishable from the right branch as to its structure. Now and again the right limb presents more connective tissue than is found in the neighboring myocardium.

The left branch is characterized by somewhat more connective tissue and is on the whole somewhat paler staining than the surrounding musculature, with its individual muscle-fibers slightly coarser and not so densely constructed, though only rarely presenting a clear central space.

The vessels of both the specialized and typical musculature present sclerosis of the intimal and mesial coats. The muscles about the vessels (especially perhaps those of the outer coat of longitudinal fibers)[4] are finer and paler than the cross-striated typical cardiac muscle-fibers and resemble those of the nodal musculature of the heart. The capillaries are everywhere engorged and in places small extravasations have occurred into the tissues, possibly signifying nothing more than ante-mortem or post-mortem stasis.

There is a good deal of connective tissue in the interventricular septum but no evidence of recent inflammation. Besides the calcified area in the upper part of the interventricular septum (in the angle of bifurcation of the auriculoventricular system and crowding in on the stem) there are isolated areas with lime deposit in a region of much connective tissue between the right and left sides of the interventricular septum and in some connective tissue of the right ventricular musculature.

The smooth muscle of the left endocardium shows more connective tissue than normally. As usual, its muscle fibers, like those about the vessels, resemble the muscle-fibers of the nodes of the heart.

The endocardium is normal, except that part of the left endocardium which covers the degenerated area where the cellular infiltration extends into it and dense connective tissue replaces the usual loose subendothelial tissue to quite an extent.

The epicardium of the posterior surface (the only epicardium on the sections) presents a thick, compact connective-tissue layer under a not inconsiderable part of the epithelium. In this layer there is (below the level of the node and stem) both a scattered and a dense cellular infiltration: that is, round cells, a few cells with larger cell body and larger vesicular nucleus, and an occasional polynuclear

4. This may be due to the direction in which they are cut. It should be noted that in this (as well as in other hearts) some of the ordinary cardiac musculature seems paler or more vacuolated than the rest owing to the direction in which the muscle-fibers are cut.

are found scattered and in masses. A most imposing focus of dense cellular infiltration is seen in the subepicardial fatty tissue immediately under the compact connective tissue layer in the lowest sections.

SUMMARY

Though the auriculonodal junction is not very powerful, it is not, apparently, inadequate, but there is an actual interruption of the continuity of the auriculoventricular structure along its course to account for the symptoms in this case; both branches of the conducting apparatus are cut off from the main stem of the auriculoventricular system and from each other. There is undoubtedly more connective tissue in the stem and more vascularity (with its attendant cellular infiltration) than is normal; and the upper ends of both limbs almost wholly and the body of the stem for about 1 mm. of its length have been demolished by a chronic, necrosing, calcifying process. In addition, there is an inconspicuous cellular infiltration at the node, as well as a more marked one of the stem and an imposing accumulation of round cells in the subepicardium; there is sclerosis throughout the coronaries and their intermuscular branches, and an increased amount of connective tissue in the ordinary ventricular musculature and some little calcific necrotic tissue.

HEART 2

HEART 2.—*Case Report.*—The heart was forwarded to Dr. L. F. Barker of Johns Hopkins Hospital by Dr. D. A. Campbell, professor of medicine, Halifax, Nova Scotia, together with the following notes: "I first saw J. C., a retired mariner, in the summer of 1905, shortly after he was admitted to an institution for the care of the aged. He was then in his ninety-fifth year. His bodily health was excellent. He was quite corpulent, but unusually active. He was generally cheery and bright in disposition. His mental faculties were well preserved. Physical examinations disclosed the existence of some enlargement of the heart, and the presence of a very loud, harsh, aortic systolic murmur. The pulse rate was 60 and quite regular. During the following winter he had a sharp attack of influenza, from which he recovered completely. During the summer of 1906 he was ill the greater part of the time, but never wholly confined to bed. The most pronounced symptoms were: mental depression, cyanosis, bradycardia (the pulse-rate varying from 10 to 40 without any irregularity), slight seizures resembling petit mal occurring very frequently some days, and Cheyne-Stokes breathing especially at night. On account of the patient's being so fat, no superficial veins could be noticed in the neck. Various remedies were tried to afford relief without much success. Finally belladonna was given in moderate doses and improvement followed. That belladonna favorably influenced the conditions was evident from the fact that when the remedy was discontinued for ten days or so most of the symptoms returned.

"He continued to keep fairly well for a period of ten months, using the belladonna the greater part of the time.

"Early in August, 1907, the symptoms returned and gradually increased in severity, causing his death a month later. The bradycardia was not so marked as before, 30 being the lowest pulse-rate noted, and the epileptiform seizures were infrequent. The more prominent terminal symptoms were: marked cyanosis, Cheyne-Stokes breathing and total disappearance of the radial pulse and cardiac impulse for periods varying from thirty to sixty seconds in duration. This very unusual modification of the pulse was noted for five days before death, and seemed to be closely connected with Cheyne-Stokes respiration. The pulse usually, but not always, disappeared during the period of dyspnea, returning during the period of apnea. No sound whatever could be heard over the cardiac area when the radial pulsations were absent."

Macroscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. Grossly the fixed heart presented no abnormality in the region of the conducting system which would account for heart-block.

Microscopic Examination of the Heart.—For microscopic examination the following blocks of tissue were excised:

1. The region of the sinu-auricular node. This block consisted of the auricular wall on either side of the sulcus terminalis of His extending posteriad as far as the interauricular septum and including a part of the superior and inferior venae cavae, and extending anteriad so as to include some of the pectinate musculature (a strip of about 0.5 cm.). Moreover, this block extended along both sides of the crest of the right auricle, thus including not only the part already described on the lateral aspect of the auricle on either side of the sulcus, but also the auricular wall on the median aspect facing the aorta. On the lateral aspect the area reached almost to the auriculoventricular groove; on the median face it extended from the crest down for about 1 cm. toward the insertion of the aorta and was a continuation, as regards its width, of the lateral portion.

2. The auriculoventricular septum, containing the auriculoventricular node, the main stem, and the beginning of the branches. The sections were cut horizontally, approximately parallel to the stem.

3. A portion of the upper ventricular septum (left side) containing a part of the left branch of the bundle of His.

The first two pieces were embedded in celloidin-paraffin and cut in sections 15 microns thick. Every fifth section was mounted and stained with Weigert's iron-hematoxylin and van Gieson's picric acid fuchsin solution. A few sections were stained with orcein. The third piece was frozen, cut, and one section stained with Scharlach R, one section stained with iron-hematoxylin and van Gieson's solution, and finally one with orcein.

Sinu-Auricular Node Region.—The sinu-auricular node is sclerotic. There are small compact masses of connective tissue between the muscle-fibers; there is more connective tissue at the cephalic than at the caudal end of the node, where the muscle-fibers are separated by looser connective tissue instead of by "wads." Even allowing for individual variation within normal limits, the increase of connective tissue in this case is so marked throughout the entire extent of the node that it may be safely stated to be abnormal. No such fibrosis is present in the musculature immediately about the node or in the other portions of the right auricle examined. In the main the nodal fibers themselves may be described as lacking in cross striations. There is, however, the typical gradual transition from nodal into ordinary auricular musculature. The nodal artery, present in this heart, shows sclerosis of the media and a slight endarteritis. Some of the

smaller branches too show these changes as do also the artery on its way to the node and some of the vessels among the ordinary cardiac musculature; occasionally a vessel presents obliterating arteritis. Some of the sections of the arteries have an outer layer of longitudinal smooth muscle-fibers arranged in small bundles.[5] Some of the nuclei of the auricular musculature are large and present uneven distribution of the chromatin and sometimes an irregular margin.[6]

Here and there in the pectinate and other ordinary cardiac musculature as well as in the specialized musculature of the Keith-Flack node, a very few cells which resemble lymphocytes are seen. They are slightly larger, have more cytoplasm, and their nuclei are more vesicular, less compact than those of lymphocytes. At one place in the nodal fat there is a small group of these cells. Larger collections of them appear in the epicardium and subepicardium; the largest at the level of the pace-making apparatus. Sometimes they are found about the subepicardial nervous elements in the neighborhood of the node. Now and again a nerve passes from the subepicardium into the sinu-auricular system. Once a ganglion cell was noticed alongside a capillary in the very center of the nodal tissue.

In general the effects of the pathological changes in the node of Keith and Flack are not yet established, and we do not venture on judging the significance of the sclerosis of this structure in this case.

Region of the Auriculoventricular System.—The connections between the auricular musculature and the node of Tawara are scanty. The node in the sections is an irregular semicircle, the diameter of which lies along the central fibrous body, and the circumference of which is almost entirely surrounded by fat tissue, thus practically separating the nodal from the auricular musculature. There is an abnormal amount of fat tissue in the region of the interauricular septum. The node contains very little connective tissue.

Cross-sections of the artery in the specialized musculature present sclerosis of the intima and the media. Some of the very small vessels have a marked diminution of their lumina; but it is impossible to say which of the two inner coats is involved. There is intimal and mesial sclerosis of the arteries in the interauricular and interventricular septa, and of the coronary artery (with atheroma). There is calcific deposit in the wall of the aorta not only near the opening of the right coronary but even nearer to the auriculoventricular system than that.[7]

As was to be expected in sections of a heart from a patient advanced in years, lipochrome granules are seen in the muscles of the auricle, ventricle, and auriculoventricular system. Some of the nuclei of both the auricular and the ventricular muscles were of the "swollen" type already described. The smooth muscle of the left endocardium presents increase of connective tissue as does the ordinary interventricular musculature especially on the left side.

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5. Such bundles of longitudinal smooth muscle-fibers are normally found in all hearts.

6. The nuclei of the swollen type mentioned above were described by Dr. A. E. Cohn in Heart, 1912, iv, 24.

7. In another heart (from a patient without data as to heart-block) though calcareous nodules were found grossly in the neighborhood of the bundle of His, microscopically these did not interfere with the continuity of the auriculoventricular system. This heart was kindly given us for examination by Dr. W. G. MacCallum.

Though the stem is long and slender, there is nowhere a break in the continuity of the auriculoventricular system.

Upper Ventricular Septum.—The piece from the upper part of the septum on the left side of the heart presents an increased amount of connective tissue in the ordinary cardiac musculature but no evidence of recent inflammation. There is also a slight increase in the connective tissue surrounding some of the vessels; all three vessel coats are sclerotic. There is no fatty infiltration of any of the musculature. The endocardium appears to be normal.

SUMMARY

While it cannot be affirmed that the slightness of the auriculonodal junction is the causative factor in this case of Adams-Stokes disease, yet it is of importance in the light of the fact that Armstrong and Mönckeberg[8] reported an exquisite case of lymphangio-endothelioma of the auriculoventricular node in which it was judged that the break between auricular and nodal musculature was the cause of the heart-block.

The condition of the blood-vessels in the aged patient may have been an accessory factor.

We should expect to find some cellular infiltration in arteriosclerotic hearts, and we do not know that there is any relation between the increase of cells in the interstitial tissue of the sinu-auricular node and of some of the ordinary auricular musculature and the clinical symptoms in this case.

It should be recalled that the interventricular septum, especially on the left side of the heart, presents an increased amount of connective tissue, and that some of the nuclei were of the "swollen" type with scattered chromatin and sometimes with irregular margin.

In brief, the most obvious explanation of the symptoms in this case is the auriculonodal break.

HEART 3

Heart 3.—*Clinical History.*—The heart was received from Dr. W. G. MacCallum, who performed the autopsy and gave us the clinical notes furnished him by Dr. M. G. Porter.

The patient was a white man aged 50. There was no history of syphilis; he had not used alcohol to excess. He had always been strong and healthy until eighteen months before exitus, when he had suffered from several attacks of syncope within a few days. Six months before his death he had a similar seizure and since that time he had frequently complained of giddiness on rising and of shortness of breath. Three days before his death he was seized, after violent exertion, with severe pain in his precordium, radiating down both arms. Subsequently he had repeated attacks of syncope and several epileptiform convulsions with attacks of bradycardia between which the pulse rhythm would be normal.

8. Armstrong and Mönckeberg: Arch. f. klin. Med., 1911, cii, 144.

The pulse-rate ranged between 110 and 25 beats per minute. He died in one of his seizures. The urine was acid, specific gravity 1.018, trace of albumin, no sugar. On the day previous to his death a distinct pericardial friction rub was heard over the body of the heart. This was again heard about an hour later ("pericarditis epistenocardia").[9]

Autopsy.—The autopsy revealed the cause of death to be rupture of the wall of the heart and consequent hemopericardium. "The pericardium was found to be filled with blood which was partly clotted: in places the clot was fully 1 cm. thick. There was a ragged hole in the anterior wall of the left ventricle about 1 cm. to the left of the vein that corresponds to the interventricular septum. Some blood clot was adherent to and extended into this ragged opening. The interventricular septum throughout its entire extent was opaque, of a grayish ochre color and was evidently necrotic. This appearance extended to within 1 cm. of the pulmonary orifice where it was sharply marked off from the adjacent normal heart wall. The wall of the left ventricle was greatly hypertrophied, but its tissue was not abnormal.

"The trabeculae were opaque and frequently broken through. In the angle between the wall of the ventricle and the septum was a hole, through which a probe was passed until it extended through the opening on the anterior surface of the heart. Both coronaries were markedly sclerotic. The anterior descending branch of the left coronary artery was completely occluded by a thrombus 3.5 cm. from its orifice. The sequence of events has evidently been: coronary sclerosis, thrombosis of the left descending branch, infarction of the interventricular septum, rupture of the heart wall, hemopericardium."[10]

Grossly, then, the heart presented an infarction of the interventricular septum including the neighborhood of the main stem of the bundle of His and its branches.

Microscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. For microscopic examination the following blocks of tissue were excised:

1. The region of the sinu-auricular node as described under Heart 2.

2. The auriculoventricular septum containing the auriculoventricular node, the main stem and the beginning of the branches.

3. The ventricular septum adjacent to Block 2 (just below it) with the posterior papillary muscle of the left side and the underlying ventricular wall and a piece of the apex.

4. A portion of the upper ventricular septum (left side) containing a part of the left branch of the bundle of His.

5. The anterior papillary muscle of the right ventricle with chordae tendineae.

6. The base of the anterior papillary muscle of the left ventricle and the underlying ventricular wall.

7. A portion of the wall of the left auricle.

Blocks 1, 2 and 4 were embedded, cut, mounted and stained like the corresponding pieces in Heart 2: piece 3 like blocks 1 and 2 above except that only every fortieth section was stained. The remaining blocks of tissue (5, 6 and 7) were embedded in celloidin-paraffin, cut 15 microns thick and two sections of each stained.

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9. Sternberg: Compt. rend. XVI Cong. internat. méd., Budapest, 1909, Section VI, Méd. Interne, p. 396; Verhandl. d. XXIX Deutsch. Cong. f. inn. Med., 1912, p. 444.

10. MacCallum: Johns Hopkins Hosp. Bull., February, 1908, No. 203, xix, 50.

Left Auricle (Block 7).—To begin with the least important block of tissue: The epicardium and adjoining fat and muscle of the wall of the left auricle present cellular infiltration (only an occasional polymorphonuclear leukocyte here). The infiltration is most marked in the epicardium and subepicardium; one small focus was seen in the endocardium. There is sclerosis of the media and intima of the vessels.

Region of the Sinu-Auricular Node (Block 1).—Unlike the portions from the interventricular septum, the block of tissue in the region of the sinu auricular node presents a comparatively slight cellular infiltration. The round cells (only a few polynuclears are to be seen) are present in the epicardium and subepicardium, to a less extent in the nodal tissue, and to still less an extent in the ordinary myocardium. There is practically no invasion of the endocardium. The area near the inferior vena cava is somewhat more involved than the rest of the ordinary cardiac musculature. The most marked infiltration in this block is found in two regions, namely: (1) in the epicardium at the level of the node and (2) about the subepicardial nervous elements and the musculature on the median face of the crest near the interauricular septum (i. e., the region of the synapse of the vagus as described by Marchand and Meyer[11]). There is a negligible increase in the amount of connective tissue in the head of the pacemaking apparatus. The nodal muscle-fibers are much coarser than those of Heart 2 and show cross-striations clearly. There is the usual gradual transition into ordinary cardiac musculature. There are a few pus cells in the media of the nodal artery. The vessels, both of the specialized and typical musculature, present sclerosis of the two inner coats. As usual, some of the sections of the arteries present an outer layer consisting of bundles of longitudinal non-striated muscles. Some of the nuclei of the musculature of this piece of tissue are of the swollen type. At one point the node encloses ordinary musculature. Here and there a nerve-fiber from the subepicardium is seen to enter the nodal structure; and in its very center a ganglion cell was observed.

It does not seem possible that the very slight cellular infiltration of the pacemaking system in this case could account for any decided influence on the functions of the heart.

Region of the Upper Auriculoventricular System (Block 2).—A very few infiltrating cells — for the most part lymphocytic, rarely polymorphonuclear — are seen in the conducting system. The right branch, and the stem (at the level of its division into two branches) and the central fibrous body near by present more pus cells than the node, the upper stem, and the left branch. Close at hand, at the basal end of the ventricular septum is an infected infarct with pus cells in the capillaries and adjoining tissues. There are pus cells elsewhere (everywhere round cells exceed the polynuclears in numbers), namely in the epicardium, subepicardial fat, auricular musculature, endocardium, tricuspid and mitral and aortic valves. But the most pronounced lesion in this block is situated in the typical ventricular musculature *near* the junctional tissue. Here the musculature has taken an unusual brown tint and many of the muscle nuclei seem to have disappeared. Indeed, some of the muscle-fibers themselves have apparently disappeared in their entirety and have been replaced by connective tissue. The remaining muscle-fibers are separated by columns and masses of pus cells and are shrunken. The central fibrous body is a little larger than normal. Practically all the vessels present intimal and medial sclerosis; such are the nodal artery, the aorta, the coronary artery, and, in brief, other vessels in the auricular and

11. Marchand and Meyer: Arch. f. d. ges. Physiol., (Pflueger's), 1912, cxlv, 401.

ventricular septa. Some of the nuclei of this block of tissue are of the swollen type. The smooth muscle of the left endocardium is normal.

Region of the Lower Auriculoventricular System and of the Terminal Arborizations (Blocks 3, 4, 5, 6).—Upper Ventricular Septum: In Block 4, that is, the piece from the upper part of the septum on the left side of the heart, there is histological evidence of the infarction just as there is in the other blocks from the interventricular septum. There is a round-celled and polymorphonuclear infiltration of the endocardium, subendocardium, the region of the left branch and especially of the ordinary ventricular musculature where many of the muscle-fibers are separated from one another by pus cells, are shrunken, and, though still showing cross-striations, are lacking in nuclei. Some of the muscle-fibers have indeed apparently quite disappeared. There is fat in both the region of the specialized and ordinary cardiac muscles.

Region of the Terminal Arborizations.—In Block 3, namely, the ventricular septum immediately below Block 2, no attempt was made to find and trace the right branch of the auriculoventricular system. The uppermost part of the posterior limb of the left branch was identified macroscopically as if for dissection and marked with India ink[12] and so found in the sections under the microscope; but as we did not follow the left branch continuously in serial sections from the stem on, we cannot assert that we have identified it. In this piece three, there is in the ordinary ventricular musculature, including that of the apex, a continuation of the same lesion (an infected infarct) as described for the other pieces from the ventricular septum, namely, pieces two and four. In that portion of the left limb which was marked with India ink there was no cellular infiltration; but ventral (anterior) of this region there is some infiltration of the endocardium and subendocardial musculature where it is not unlikely that the left branch is involved. Similarly there is infiltration of the endocardium and subendocardial musculature in the apex. On the other hand there is no cellular infiltration of the posterior papillary muscle of the left side. Many of the cells under the epicardium and endocardium of the right and left ventricle and of the posterior papillary muscle are broad and pale and vacuolated; some of these may be Purkinje cells, possibly some Purkinje-like because of the direction in which they are cut and others perhaps because of a Purkinje-cell variety of muscle atrophy.[13] Some of the nuclei of the cells are of the swollen type with unevenly distributed chromatin and irregular margins. The coats of the blood-vessels present an increased amount of connective tissue.

The anterior papillary muscle and chordae tendineae of the right side (Block 5) are normal except for some nuclei of the swollen type; the base of the anterior papillary muscle of the left side and underlying ventricular wall (Block 6) present pus cells in the epicardium and subepicardium and sclerosis of the two inner coats of the vessels.

In short, an almost negligible number of lymphocytic and a still lesser number of polymorphonuclear cells have invaded the auriculoventricular system, and that is all: therefore from an anatomical standpoint the conducting mechanism is in a condition to functionate, but from a physiological viewpoint the sclerosis of its vessels and the presence of even but a few pus cells in the junctional tissue

12. According to Nagayo [in Beiter. z. path. Anat. u. z. allg. Path. (Ziegler's) 1909, xlv, 295] the stripes seen macroscopically are due not only to the presence of the branches of the conducting system, but also to bundles of smooth muscle fibers.

13. Cohn and Lewis: Heart, 1912, iv, 24.

and finally the neighboring infected infarct suggest an inefficiency of circulation and an abnormal condition sufficient to account for heart-block.

DISCUSSION

The sclerosis of the coronary arteries and its branches in these three aged patients suggests that an inadequate circulation played a part in the production of the symptoms of Adams-Stokes syndrome in all three.

As was expected in arteriosclerotic hearts, some increase of the cells in the interstitial tissue was observed, but we do not know what may have been its significance in these cases.

If these are three cases of heart-block, as is suggested by the Adams-Stokes syndrome, then they form a series, Case 1 with an absolute break between the stem at its bifurcation and the two limbs right and left, Case 2 with its inadequate auriculonodal junction and Case 3 without a break in the continuity of the auriculoventricular system.

In the first case described, a lesion to account for heart-block was seen: there was complete transverse destruction of the tissue of the bundle of His. In the second case, it is impossible to state to what extent the sclerosis of the sinu-auricular node and the fact that the auriculonodal junction is slight, affected the functions of the heart. And in the other case, though histological investigation brought to light no such lesion of the auriculoventricular system (i. e., partial or complete break in its continuity), as has been frequently regarded the ultimate anatomical basis of the affection, yet the findings are indicative of such a disturbed circulation and abnormal condition in the neighborhood of the conducting apparatus as might profoundly influence its functional capacity.

SUMMARY

1. The hearts of three patients with Adams-Stokes syndrome presented, the first a calcific mass in the region of the auriculoventricular bundle, the second no gross lesion across the conducting system to account for the symptoms, the third infarction of the ventricular septum.

2. The first patient is reported to have denied a syphilitic history, in the second case the past history is not given, and the third patient is said to have had no lues. All three were well along in years with some sclerosis of the vessels and some increase of cells in the interstitial tissues.

3. The most noteworthy lesions in each case were: in the first, an absolute break in the course of the stem of the auriculoventricular sys-

tem; in the second, the slight auriculonodal junction and a sclerosis of the sinu-auricular node; in the third, an infected infarct of the ordinary ventricular musculature near the junctional tissue.

4. The possibility of insufficient circulation and of a lesion in the ordinary ventricular musculature as agents in the production of heart-block, are suggested.

We are greatly indebted to Dr. W. G. MacCallum and Dr. L. F. Bishop for the privilege of describing these cases and to Dr. A. E. Cohn for his valuable suggestions.

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THE MECHANISM OF ANAPHYLATOXIN FORMATION.

Studies on Ferment Action. XV.*

By JAMES W. JOBLING, M.D., and WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The attention that the problems of anaphylaxis and of anaphylatoxin formation have received during the past few years has led to two antagonistic conceptions as to the nature of the mechanism of intoxication.

Friedberger (1) believes that the phenomena are due to an intoxication by protein split products, placing the matrix of the poison in the foreign protein, or antigen, introduced. Doerr (2) regards them as physical and as resulting from colloidal changes in the serum, and denies the possibility of enzymotic protein cleavage as having any direct bearing on the process of intoxication. Keysser and Wassermann (3), Ritz and Sachs (4), and Bordet (5) are more or less committed to the physical theory, although they do not consider it necessary to go as far as Doerr in denying the splitting of the proteins, merely placing the matrix of the toxin in the homologous serum, without definite agreement as to the nature of the colloidal changes that are supposed to occur.

The production of the so called anaphylatoxin *in vitro* by Friedemann (6) and by Friedberger and Mita (1), by the interaction of complement, antigen, and amboceptor, has been greatly simplified. Friedberger himself found the amboceptor superfluous. Almost any adsorbing substance has been used as an antigen; such as kaolin (Keysser and Wassermann (3)), agar (Besredka and Ströbel (7), and Bordet (5)), "*Kieselguhr*" (Doerr (2)), starch (Nathan (8)), waxes and fats from bacilli (Leschke (9)), besides the true antigens, which latter need not be specific, for they are still effective after boiling. The experiments of Doerr and Russ (2), Kraus (10), Seitz (11), Aronson (12), and Friedberger have shown that complement is not essential, for toxic sera can be produced from inactivated serum, although not with the same regularity as from active serum.

If, then, the antigen-antibody hypothesis has failed to explain the accumulated facts, how are we to interpret the toxicity of the so called anaphylatoxins? The evidence still seems strong that the

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toxic substances are cleavage products of proteins, or, at any rate, proteins so altered that they are capable of being split in the organism; for Friedberger does not claim that the entire toxic effect of anaphylatoxin is due to preformed split products. The arguments made against the protein-intoxication conception, such as the time element and the minute amount of substance necessary, are not convincing, for we know that ferment action may be very rapid; and as far as the quantity of substrate is concerned the argument fails if we place the matrix of the poison in the serum proteins themselves. There is some reason to believe, however, that if ferment action is the basis of anaphylactic shock, these ferments may have a much wider range of action than merely on the introduced protein.

Doerr (2) assumes that guinea pig serum is normally toxic, but that the toxicity is held in abeyance by some unknown substance which is adsorbed during the anaphylatoxin formation. Ritz and Sachs (4) suggest that during adsorption alteration occurs in the serum resulting in the formation of toxic substances. In a measure, both of these views are probably correct, although in neither of the papers in which they are put forward are there to be found suggestions as to the nature of the adsorbed substances. Doerr calls attention to the marked changes in coagulation as manifestations of the intoxication and suggests a causal connection. We, too, feel certain that this phase warrants a more detailed study.

Granting tentatively that anaphylaxis and anaphylatoxin intoxications are due to protein split products, we come to a consideration of the matrix. That the antigen used in producing anaphylatoxin is not split, has been convincingly shown by Donati (13) who noted that organisms so used retained their antigenic properties; while Neufeld and Dold (14) observed that living bacteria produced anaphylatoxin more easily than killed organisms. The fact that the same antigen can be used repeatedly would also tend to confirm this view. The demonstration of dialyzable split products occurring in anaphylatoxin (Pfeiffer and Mita (15)) gives no clew to the origin of the cleavage products; and inasmuch as we have recently (16) shown that normal serum will autolyze rapidly when the antiferments are lowered or removed, there is reason to believe that small amounts of split products may occur in anaphylatoxin. Total non-coagulable nitrogen determinations made according to the method of Folin show, however, that whatever splitting takes place in the usual methods of anaphylatoxin formation is very small. The production of anaphylatoxins by means of kaolin, agar, starch, waxes, and infusorial earth leaves no room for doubt that the matrix must reside in the serum.

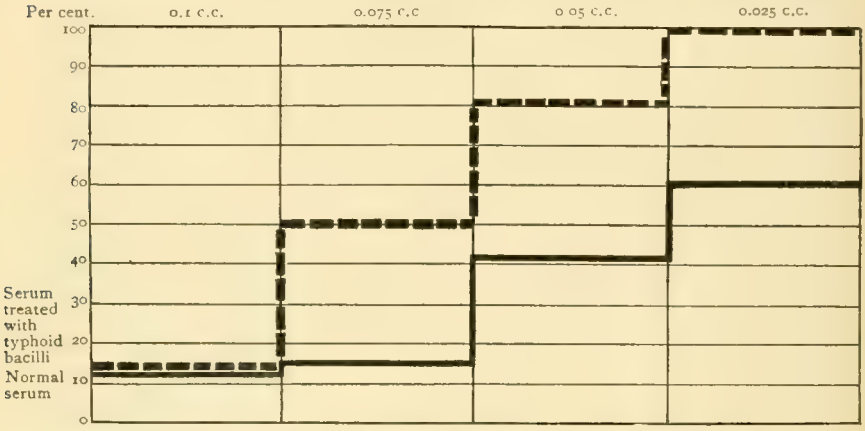
In a recent paper (16) we have demonstrated the lipoidal nature of serum antitrypsin and have shown that the inhibition is due to

unsaturated fatty acids and esters (17). We have also shown (18) that by extracting the serum with chloroform or ether, thereby removing the antitrypsin, or by saturating the free bonds with iodin, or by acidulating and filtering the serum, the serum becomes highly toxic, this toxicity depending on three factors; *i. e.*, changes in coagulation, exposure of serum proteins, and autolysis *in vitro* of the exposed proteins. Under these conditions the serum becomes from five to ten times as toxic as the ordinary anaphylatoxin, the exact minimum lethal dose depending on the amount of lipoids removed and the state of autolysis. The increase in toxicity is accompanied by a constant increase in the total non-coagulable nitrogen as determined by the Folin method, until a period of digestion is reached when the serum is no longer toxic. The mere inactivation of serum antitrypsin by heat or acid is not sufficient to secure a toxic serum, possibly because the lipoids remain in the serum and are rendered active again on reinjection. From the serotoxin which we have prepared we have isolated toxic split products, thermostabile, and lethal in doses of about 0.01 of a cubic centimeter per gram weight of guinea pig when made up to the original serum volume.

The fatty acids and their salts are easily adsorbable substances (19), and we might therefore expect that under proper quantitative conditions it would be possible to adsorb these protective lipoids from serum without affecting the other constituents. We have therefore examined the effect of the various adsorbing substances which have been found to produce typical anaphylatoxin, on the serum antitrypsin of fresh guinea pig serum and other sera. Our experiments have been made by incubating the mixture for three hours and then testing the antitryptic effects directly or after permitting the mixture to remain over night in the ice chest. In the charts illustrating these experiments the antitryptic value of the untreated serum is shown in the heavy black lines. Inasmuch as the ordinary Fuld-Gross technique for antitrypsin determination leads to serious errors in the reading, we have, in every instance, determined the total nitrogen digestion by the method of Folin, which we have described in our former papers.

EXPERIMENTS WITH TYPHOID ANAPHYLATOXIN.

Fresh guinea pig serum was mixed with typhoid bacilli in the proportion of two cubic centimeters of serum to one agar slant of bacilli and the mixture was incubated for three hours. The mixture was then placed in the ice chest over night. As will be seen from text-figure 1, there was a marked decrease in the antitryptic value of the serum so treated. There was no increase in the non-coagulable nitrogen of the serum. A portion of the mixture was



TEXT-FIG. 1. Adsorption of serum antitrypsin from guinea pig serum by typhoid bacilli.

centrifuged until it became clear, and was then injected intravenously into guinea pigs to determine its toxicity. Two cubic centimeters caused immediate death of a guinea pig weighing 210 grams, with all the evidences of anaphylaxis. The experiments have been repeated several times with similar results.

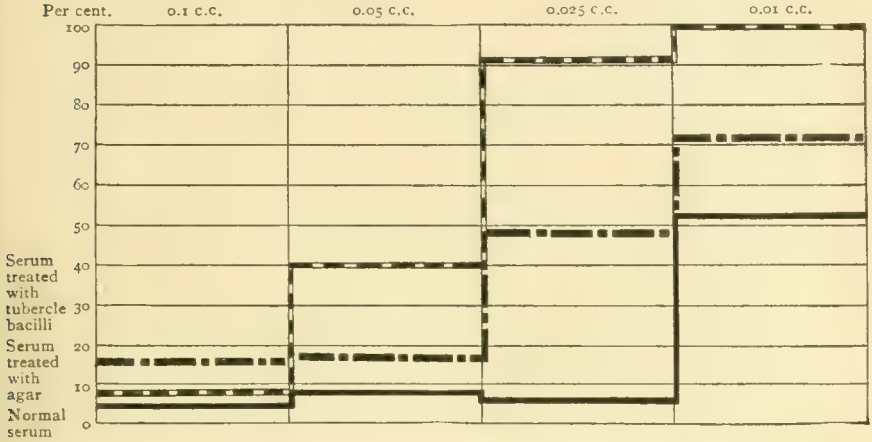
EXPERIMENT WITH AGAR AND TUBERCLE BACILLI ANAPHYLATOXIN.

A similar experiment was made with dried tubercle bacilli and also with a small amount of solid agar. As will be seen from text-figure 2, tubercle bacilli have somewhat, and the agar has greatly lessened the serum antitrypsin. It is interesting to recall that the production of anaphylatoxin from tubercle bacilli has always been somewhat difficult, and that Shibayama (20) found

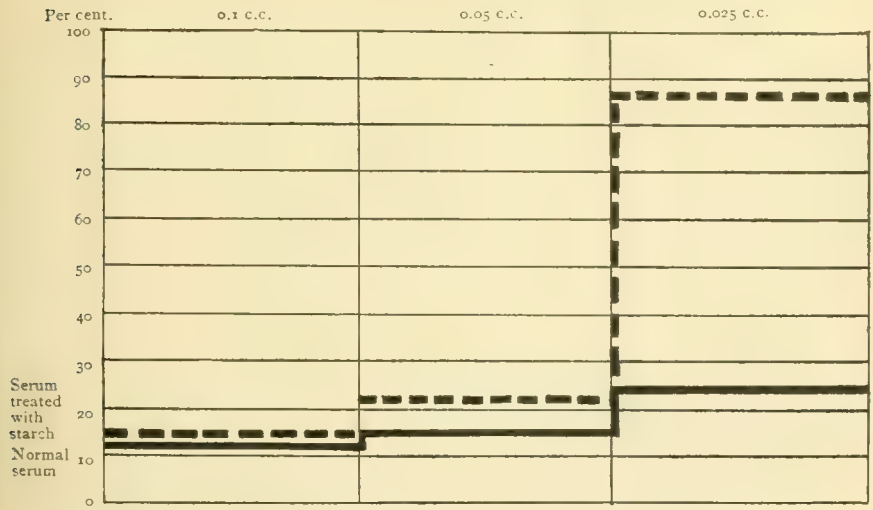
that by extracting the fats from the bacilli the result was much more constant.

ANAPHYLATOXIN FROM STARCH.

The next experiment was made with starch as an adsorbent. The data from which text-figure 3 has been made will be found in protocol I. In this experiment a large amount of starch paste



TEXT-FIG. 2. Adsorption of serum antitrypsin by agar and tubercle bacilli.



TEXT-FIG. 3. Adsorption of serum antitrypsin by starch.

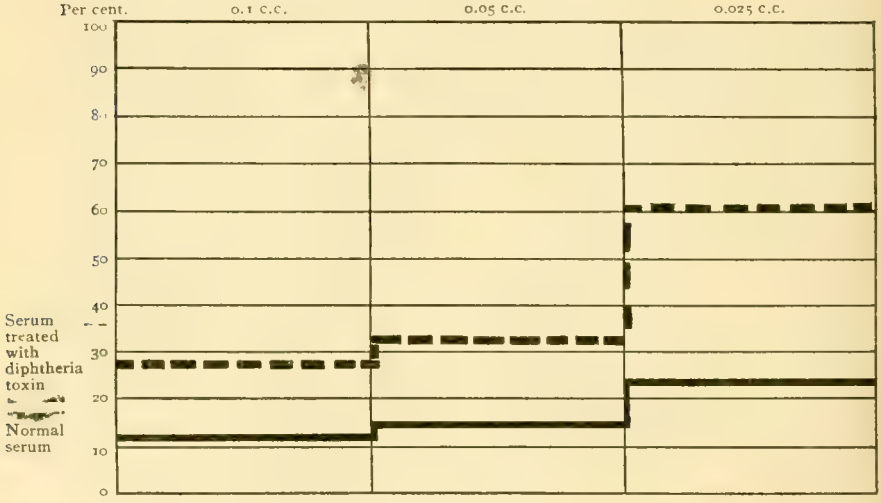
adsorbed less of the antitrypsin than a smaller amount, which is in accord with the actual toxic effects observed by Nathan (8).

The following experiment illustrates the method that we have used in this series of experiments.

2 c.c. of fresh guinea pig serum were mixed with each of the following substances:

1 c.c. of diphtheria toxin (minimum lethal dose = 0.0025 c.c.).
0.1 c.c. of diphtheria toxin (minimum lethal dose = 0.0025 c.c.).
0.2 gm. of kaolin.
0.05 gm. of kaolin.
0.1 c.c. of starch paste.
0.05 c.c. of starch paste.
0.25 gm. of agar (semisolid) active serum.
0.25 gm. of agar (semisolid) inactivated serum.

The mixtures were incubated for three hours, placed in the ice chest over night, and the next morning each tube was diluted one tenth to twenty cubic centimeters with normal salt solution. The



TEXT-FIG. 4. Effect of diphtheria toxin on serum antitrypsin.

kaolin, starch, and agar sera were then centrifuged, and the supernatant serum was mixed with trypsin in the proportion shown in protocol I, incubated for thirty minutes, and two cubic centimeters of 1 per cent. casein solution were then added. After digestion was complete, the undigested casein and serum proteins were pre-

PROTOCOL I.

No. of tube.	1 per cent. casein solution.	Trypsin.	Serum 1:10 + diphtheria toxin.		Serum 1:10 + kaolin.		Serum 1:10 + starch.		Serum 1:10 + agar 0.25 gm.		Normal guinea pig serum 1:10.	Total nitrogen digestion.	Digestion.	
			1 c.c. toxin.	0.1 c.c. toxin.	0.2 gm. kaolin.	0.05 gm. kaolin.	0.1 c.c. starch.	0.05 c.c. starch.	Active.	Inactive 54° C.				
1	2 c.c.	0.0 c.c.										Trace	0%	
2	2 c.c.	0.2 c.c.										1.66 mg.	100%	
3	2 c.c.	0.2 c.c.	1.0 c.c.									0.45 mg.	27%	Diphtheria toxin.
4	2 c.c.	0.2 c.c.	0.5 c.c.									0.55 mg.	37%	
5	2 c.c.	0.2 c.c.	0.25 c.c.									1.0 mg.	60%	
6	2 c.c.	0.2 c.c.		1.0 c.c.								0.26 mg.	15%	
7	2 c.c.	0.2 c.c.		0.5 c.c.								0.3 mg.	17%	
8	2 c.c.	0.2 c.c.		0.25 c.c.								0.8 mg.	47%	
9	2 c.c.	0.2 c.c.			1.0 c.c.							0.2 mg.	12%	Kaolin.
10	2 c.c.	0.2 c.c.			0.5 c.c.							0.2 mg.	12%	
11	2 c.c.	0.2 c.c.			0.25 c.c.							0.3 mg.	17%	
12	2 c.c.	0.2 c.c.				1.0 c.c.						0.3 mg.	17%	
13	2 c.c.	0.2 c.c.				0.5 c.c.						0.74 mg.	44%	
14	2 c.c.	0.2 c.c.				0.25 c.c.						0.93 mg.	56%	
15	2 c.c.	0.2 c.c.					1.0 c.c.					0.13 mg.	8%	Starch.
16	2 c.c.	0.2 c.c.					0.5 c.c.					0.48 mg.	28%	
17	2 c.c.	0.2 c.c.					0.25 c.c.					0.7 mg.	42%	
18	2 c.c.	0.2 c.c.						1.0 c.c.				0.26 mg.	15%	
19	2 c.c.	0.2 c.c.						0.5 c.c.				0.36 mg.	21%	
20	2 c.c.	0.2 c.c.						0.25 c.c.				1.43 mg.	86%	
21	2 c.c.	0.2 c.c.							1.0 c.c.			Lost	Lost	Agar.
22	2 c.c.	0.2 c.c.							0.5 c.c.			0.35 mg.	20%	
23	2 c.c.	0.2 c.c.							0.25 c.c.			0.83 mg.	50%	
24	2 c.c.	0.2 c.c.								1.0 c.c.		0.25 mg.	15%	
25	2 c.c.	0.2 c.c.								0.5 c.c.		0.37 mg.	21%	
26	2 c.c.	0.2 c.c.								0.25 c.c.		1.25 mg.	75%	
27	2 c.c.	0.2 c.c.									1.0 c.c.	0.21 mg.	12%	Normal.
28	2 c.c.	0.2 c.c.									0.5 c.c.	0.24 mg.	15%	
29	2 c.c.	0.2 c.c.									0.25 c.c.	0.4 mg.	24%	
30	0 c.c.	0.0 c.c.	0.05 c.c. diphtheria toxin									0.1 mg.		

All tubes were made up to 5 c.c. with normal saline solution.

cipitated by salt and acid, boiled, filtered through kaolinized hard paper filters, and nitrogen determinations made on the filtrate. The nitrogen determined for each tube in reference to the total digestion of the casein gives the exact percentage of digestion.

Text-figure 4 shows the effect of diphtheria toxin on the serum antitrypsin and illustrates the digestion in tubes 3, 4, and 5 of protocol I. The amount of non-coagulable nitrogen in 0.05 of a cubic centimeter of diphtheria toxin (the largest amount used) was found to be 0.1 of a milligram.

It will be noted that kaolin had not affected the antitrypsin when the larger amount was used, but the smaller amount (0.05 of a gram) had adsorbed a large amount. Our experience has been that agar and starch and bacteria are more effective, as a rule, than kaolin, which is only effective in a certain definite quantitative relation. In the series with agar the inactive serum also shows adsorption of the antitrypsin. We have found it on the whole more difficult to secure adsorption from the inactivated serum than from the active, corresponding to the condition which obtains with chloroform extraction. It has been demonstrated repeatedly, however, that even inactivated sera will produce anaphylatoxins although not so constantly as active sera. The relation of the toxicity of normal guinea pig serum to its antiferment content has already been discussed in a previous paper in which it was shown that the toxicity is proportionate to the degree of removal of the antitrypsin, the serum becoming more toxic with decreasing antitryptic activity. In our present work with bacteria, agar, starch, etc., we have found that the same conditions apply, the agents which absorb most antitrypsin producing the most toxic sera. For these reasons we shall not describe the numerous experiments made in the course of the work.

EFFECT OF TOXINS ON SERUM ANTITRYPSIN.

When we consider the effect produced by true toxins we must, of course, seek a different explanation than one of mere adsorption. Friedberger and Mita (21) demonstrated that an active toxin could be produced by incubating tetanus toxin, diphtheria toxin, and cobra venom with fresh guinea pig complement. Their efforts met

with least success with the cobra venom preparation. They interpreted their results as indicating that the complement had split the toxins to still more toxic products which killed with typical anaphylactic symptoms. In view of the exhaustive work that has been done in the differentiation of the toxins and their components this view as to the unity of toxin action seems at least unreasonable. It has been demonstrated that the toxins have a special affinity for lipoidal substances (de Waele (22), Raubitschek (23)). Whether this depends on the presence of unsaturated fatty acids has not been determined and we are now conducting experiments in this direction. If, then, these lipoidal bonds are first saturated by a toxin we should expect that the trypsin subsequently added would be bound to a lessened degree, since the lipoids are the same in both cases. It is, of course, quite probable that the phenomenon is a purely physical one, but it is interesting to note in this connection the close parallelism shown by the behavior of the toxins and the enzyme in their relation to these lipoids. In protocol I the effect of diphtheria toxin has already been noted.

PROTOCOL II.

No. of tube.	1 per cent. casein solution.	Trypsin.	Serum normal 1 : 10.	Serum 1 : 10 + cobra venom.	Total nitrogen digestion.	Digestion.	
1	2 c.c.	0.0 c.c.			0.0 mg.	0%	
2	2 c.c.	0.2 c.c.			1.65 mg.	100%	
3	2 c.c.	0.2 c.c.	1.0 c.c.		0.28 mg.	17%	Normal.
4	2 c.c.	0.2 c.c.	0.75 c.c.		0.41 mg.	25%	
5	2 c.c.	0.2 c.c.	0.5 c.c.		0.71 mg.	43%	
6	2 c.c.	0.2 c.c.	0.25 c.c.		1.11 mg.	67%	
7	2 c.c.	0.2 c.c.	0.1 c.c.		1.2 mg.	72%	
8	2 c.c.	0.2 c.c.		1.0 c.c.	0.31 mg.	18%	Cobra venom.
9	2 c.c.	0.2 c.c.		0.75 c.c.	0.6 mg.	36%	
10	2 c.c.	0.2 c.c.		0.5 c.c.	0.83 mg.	50%	
11	2 c.c.	0.2 c.c.		0.25 c.c.	1.2 mg.	72%	
12	2 c.c.	0.2 c.c.		0.1 c.c.	1.5 mg.	0.67%	

All tubes were made up to 5 c.c. with normal saline solution.

In our next experiment (protocol II) the results showed that cobra venom[1] also lowered the antitryptic value only to a small extent, a result to be expected from the experiments of Friedberger

[1] The cobra venom was kindly furnished us by Dr. Noguchi.

and Ito, who found it difficult to secure toxic serum in this way, having only one acute death in their series.

The reason why quantitative differences should so markedly influence anaphylatoxin formation is not clear, although we have noted frequently that large amounts of bacteria, kaolin, or starch will adsorb very little of the antitrypsin, while smaller amounts will be effective. That such a condition should be true for bacteria might seem reasonable, for when large numbers are incubated a proportion may die and undergo solution, due to the lipolytic effect of the serum, while the bacterial antiferments, together with the antiferments previously adsorbed from the serum will be thrown back into solution, rendering it more antitryptic than before. That lessened toxicity when larger amounts of bacteria are used should be due to complete protein splitting to atoxic products is out of the question, for even with a concentrated leucoprotease solution Jobling and Strouse (24) found that it required several days to secure highly toxic products from typhoid bacilli.

To show the effects of various amounts of bacteria when incubated with fresh serum, we have carried out the experiment shown in protocol III. The technique was the same as before, with the exception that only one cubic centimeter of serum was used for each mixture, the final dilution being made to ten cubic centimeters, instead of twenty. The number of bacteria used is, of course, not absolute, the measure being a large platinum loop, the amounts used being 4, 2, 1, and ½ loops to each cubic centimeter. From the results it will be seen that the staphylococci are effective only in the larger amounts, while typhoid bacilli give the most marked effects in the one and two loop mixtures, the four loop mixture being less effective.

If serum antitrypsin is adsorbed by the bacteria used in the preparation of anaphylatoxin we should expect that such bacteria would become more resistant to tryptic digestion than untreated bacteria. In order to determine this point the following experiment was carried out.

Typhoid bacilli which had been incubated with fresh guinea pig serum (complement) in the preparation of anaphylatoxin were centrifuged, washed, and dried by grinding in an agate mortar. The dry weight was 30 mg. A similar amount

PROTOCOL III.

No. of tube.	1 per cent. casein solution.	Trypsin.	Serum 1:10 + staphylococci. Loops.				Serum + typhoid. Loops.				Normal serum 1:10.	Total nitrogen digestion.	Digestion.	
			4	2	1	½	4	2	1	½				
1	2 c.c.	0.0 c.c.										Trace	0%	
2	2 c.c.	0.2 c.c.										1.54 mg.	100%	
3	2 c.c.	0.2 c.c.									1.0 c.c.	0.15 mg.	10%	Normal.
4	2 c.c.	0.2 c.c.									0.5 c.c.	0.41 mg.	26%	
5	2 c.c.	0.2 c.c.									0.25 c.c.	0.8 mg.	52%	
6	2 c.c.	0.2 c.c.	1.0 c.c.									0.35 mg.	22%	Staphylococci.
7	2 c.c.	0.2 c.c.	0.5 c.c.									0.72 mg.	47%	
8	2 c.c.	0.2 c.c.	0.25 c.c.									1.3 mg.	84%	
9	2 c.c.	0.2 c.c.		1.0 c.c.								0.26 mg.	16%	
10	2 c.c.	0.2 c.c.		0.5 c.c.								0.32 mg.	21%	
11	2 c.c.	0.2 c.c.		0.25 c.c.								0.85 mg.	55%	
12	2 c.c.	0.2 c.c.			1.0 c.c.							0.22 mg.	14%	
13	2 c.c.	0.2 c.c.			0.5 c.c.							0.48 mg.	31%	
14	2 c.c.	0.2 c.c.			0.25 c.c.							1.4 mg.	90%	
15	2 c.c.	0.2 c.c.				1.0 c.c.						0.3 mg.	20%	
16	2 c.c.	0.2 c.c.				0.5 c.c.						0.35 mg.	22%	
17	2 c.c.	0.2 c.c.				0.25 c.c.						1.4 mg.	90%	
18	2 c.c.	0.2 c.c.					1.0 c.c.					0.35 mg.	22%	Typhoid.
19	2 c.c.	0.2 c.c.					0.5 c.c.					0.62 mg.	40%	
20	2 c.c.	0.2 c.c.					0.25 c.c.					0.83 mg.	54%	
21	2 c.c.	0.2 c.c.						1.0 c.c.				0.43 mg.	26%	
22	2 c.c.	0.2 c.c.						0.5 c.c.				0.47 mg.	30%	
23	2 c.c.	0.2 c.c.						0.25 c.c.				1.1 mg.	71%	
24	2 c.c.	0.2 c.c.							1.0 c.c.			0.5 mg.	32%	
25	2 c.c.	0.2 c.c.							0.5 c.c.			0.4 mg.	25%	
26	2 c.c.	0.2 c.c.							0.25 c.c.			0.9 mg.	58%	
27	2 c.c.	0.2 c.c.								1.0 c.c.		0.31 mg.	20%	
28	2 c.c.	0.2 c.c.								0.5 c.c.		0.45 mg.	27%	
29	2 c.c.	0.2 c.c.								0.25 c.c.		0.55 mg.	36%	

All tubes were made up to 5 c.c. with normal saline solution.

of dried untreated typhoid bacilli was used as a control. They were each suspended in 3 c.c. of normal salt solution and 10 mg. of sodium carbonate, and 0.1 c.c. of trypsin solution was added to each. The trypsin was of such strength that 0.1 c.c. would digest 2 c.c. of a 1 per cent. casein solution in two hours. The tubes were kept in the incubator over night. The next morning the mixtures were acidified, boiled, filtered through kaolinized hard filters, and nitrogen determinations (Folin) were made to determine the amount of digestion.

No. of tube.	Original typhoid.	Serum-treated typhoid.	Total nitrogen digestion.	Per cent. digestion.
1	3 c.c.	0 c.c.	1.5 mg.	50
2	0 c.c.	3 c.c.	0.375 mg.	12
3	3 c.c.	Total nitrogen = 3 mg.		

The digestion of the untreated bacteria is, therefore, over four times as great as of those bacteria which have adsorbed antitrypsin from the serum.

With staphylococci this difference is not so great, but in the experiments which we have made, the digestion of the untreated bacteria is always somewhat greater than of those which have been treated with fresh serum. We have stated that one can adsorb the antitrypsin from the inactivated serum almost as well as from the active guinea pig serum, although the process seems to be slower. That even inactivated sera will form anaphylatoxins has been shown before (2, 10, 11, 12). From human serum it is much more difficult to remove the antiferment by adsorption. Horse serum antitrypsin we have found almost as easily adsorbable as the guinea pig antitrypsin.

DISCUSSION.

The interpretation of our results is, we believe, simple. The serum proteins are normally prevented from digestion intravitally by the presence of protective lipoids,—unsaturated fatty acids, but when these are removed the proteolytic ferment of the serum can rapidly split the proteins; the exposure of the proteins by partial removal of the antitrypsin through adsorption, or complete removal by means of the lipoidal solvents renders the serum highly toxic to the homologous animal. The recent work of Dold and his coworkers (25) affords a complete confirmation of this view. They state that anaphylatoxins could not be obtained *in vivo* or *in vitro*

with bacteria which had been treated with oils, with bile, or with cholesterol, but that the bacteria had not lost their power to act as antigens, and still contained active endotoxins.

Bacteria treated in this manner have, of course, been saturated with lipoids and can no longer take up more when placed in contact with fresh serum. If the bacteria had been split, as Friedberger contends, then it would be impossible to secure toxic effects *in vivo*, and their antigenic properties would be destroyed. We have repeated the experiments of Dold and his co-workers and have never noted any adsorption of antitrypsin from fresh guinea pig serum by bacteria so treated; neither did the serum so treated become toxic.

The views of Friedberger are, therefore, untenable in that they would make one uniform toxic agent responsible for the manifold toxic effects observed in disease. While split products of proteins (primary proteoses) whether from a foreign source or from the organism's own proteins, are no doubt the causative agents in many instances, the mechanism of their production is unquestionably different from the one supposed by Friedberger. From the complexity of the protein molecule, we should expect that even the higher cleavage products would show differences in their toxic, as they do in their antigenic properties; and the unfailing uniformity of the toxic effects of the anaphylatoxins, together with the lack of antigenic properties, is one of the strongest arguments that the substrate from which the toxic substances are split is the same in every case. That Friedberger (26) himself seems to realize that the position which he has taken is untenable is indicated in his recent work with kaolin, the toxic effects of which he found could be prevented by previous treatment with serum. He says: "The toxicity of kaolin is not due to mechanical injury; it is probably due to the adsorption of certain substances from cells fundamental to the life of the organism."

CONCLUSION.

1. The unsaturated lipoids (serum antitrypsin) can be adsorbed from guinea pig serum, rabbit serum, and horse serum by kaolin, starch, agar, and bacteria.

2. Diphtheria toxin and cobra venom also reduce the serum antitrypsin, possibly because of their affinity for lipoids.

3. Anaphylatoxins represent sera rendered toxic by partial removal of serum antitrypsin.

4. The matrix of the protein split products lies in the serum proteins so exposed.

5. The amount of removal of serum antitrypsin depends on definite quantitative relations; very large amounts and very small amounts of adsorbing substances are least effective (kaolin, starch, and bacteria).

6. Bacteria previously treated with serum or with oils do not adsorb serum antitrypsin.

7. Bacteria treated with serum become more resistant to the action of trypsin.

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THE REMOVAL OF CALCIUM FROM THE BLOOD BY DIALYSIS IN THE STUDY OF TETANY.*

By W. G. MacCALLUM, M.D., R. A. LAMBERT, M.D., and KARL M. VOGEL, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

On the appearance of the paper by Abel, Rowntree, and Turner[1] describing a method by which diffusible substances might be removed from the circulating blood by dialysis, it occurred to us that we might attack the question of the relation of calcium salts to tetany in a new way.

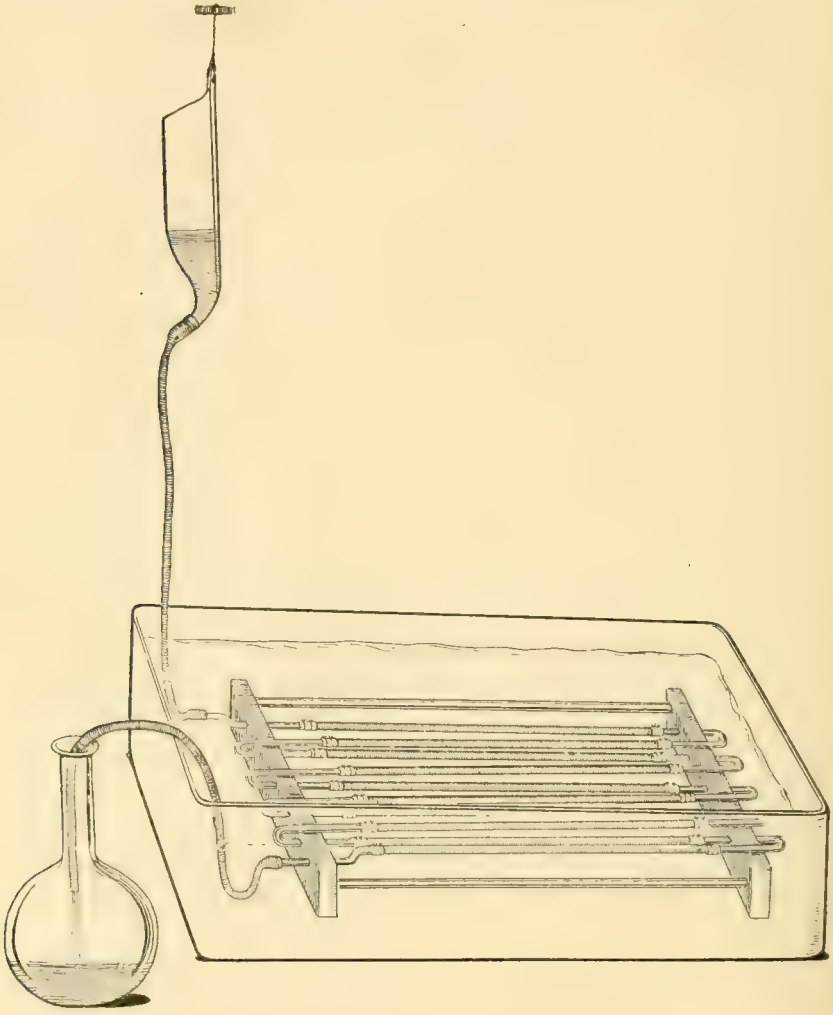
At first we thought that we might be able to produce tetany by mechanically removing calcium from the blood by allowing it to circulate through celloidin tubes immersed in an isotonic solution containing no calcium. We therefore constructed a machine somewhat like Abel's, and made a number of experiments with this object in view, using a fluid put together according to Abderhalden's analysis of the inorganic constituents of the dog's blood, but without calcium. We varied this fluid by substituting phosphates for chlorides, with the idea that by removing chlorides also we might still further reduce the proportion of soluble calcium. We used defibrinated blood instead of salt solution in starting the current, and finally defibrinated all the animal's blood. The results were practically negative, and we abandoned the method because it seemed that we could attain our end better in another way without exposing the animal to an operation which must be prolonged over many hours.

We adopted a method which consisted in passing a quantity of defibrinated blood from another dog through a modified dialyzing apparatus (text-figure 1) for many hours until, as we could ascer-

* Received for publication, June 2, 1914.

[1] Abel, J. J., Rowntree, L. G., and Turner, B. B., *Jour. Pharmacol. and Exper. Therap.*, 1914, v, 275.

tain by chemical analysis, the amount of calcium was greatly reduced and had passed over into the dialyzing fluid. It was then



TEXT-FIG. 1. Dialysis apparatus of celloidin tubes in series.

proposed to run the blood into the veins of a normal animal after an equal amount had been removed from the carotid artery, but even before this was done, it was thought best to ascertain the effect of

the dialyzed blood upon the nerves of an isolated extremity by perfusion, according to the method previously employed[2] in studying the blood of animals in tetany. A number of experiments made in this way gave the following results.

Dog 1,420.—Normal extremity. Perfusion started at 3.50 P. M. with blood dialyzed against calcium-free fluid.

	2.00	3.30	3.50	4.00	4.10	4.20	4.40	4.50
KC[3]	0.2	0.05	Perfusion started	0.05	0.05	0.05	0.1	0.2
KO	—[4]	—		0.6	1.8	1.2	1.6	2.4
AC	0.5	1.0		0.2	0.5	0.6	0.4	1.2
AO	1.4	0.4		0.5	0.5	0.6	0.4	1.2

Blood used in perfusion contained calcium per 1,000 c.c. as follows:

Before dialysis.	After dialysis.	After perfusion.
0.085	0.0465	0.080

Dog 1,421.—Normal isolated extremity. Perfusion started at 4.15 P. M. in the same way.

	3.50	4.00	4.15	4.20	4.30	4.45	5.00	5.15	5.30
KC	0.4	0.6	Perfusion	0.2	0.4	0.4	0.4	0.4	0.4
KO	—	—		1.6	2.8	1.8	1.8	2.6	1.6
AC	1.0	1.6		1.2	1.0	1.0	1.2	1.0	1.0
AO	1.2	2.0		1.2	1.0	1.0	1.0	0.8	1.0

Dog 1,427.—Normal leg perfused first with normal then with dialyzed blood.

	8.45	9.00	9.30	9.45	9.50	10.00	10.10	10.15	10.20	10.30	10.40	10.50	11.00	11.10	11.20	11.30	11.35	11.50
KC	0.3	0.4	Perfusion complete Normal blood	0.6	0.8	0.8	1.0	1.0	0.6	0.6	0.6	0.6	0.8	0.8	1.0	1.0	2.0	1.2
KO	—	—		—	—	—	—	—	4.4	3.6	4.0	3.4	3.4	—	—	—	—	—
AC	1.6	1.6		2.4	2.4	2.4	2.8	2.4	2.0	2.0	2.0	2.0	2.2	2.2	2.4	3.4	3.4	4.0
AO	1.8	2.4		—	4.6	4.6	3.2	2.6	2.0	2.0	2.0	2.0	2.0	1.4	2.4	2.2	2.6	4.0

Dialyzed blood — Normal blood

[2] MacCallum, W. G., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv, 941

[3] KC = kathode closing; AO = anode opening, etc.

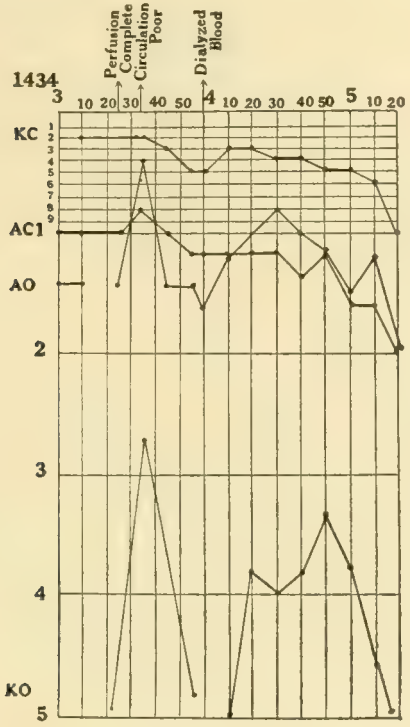
[4] In all the tables — indicates negative up to 5 milliamperes.

It will be seen from these tables that the results of perfusion with dialyzed blood were not especially striking, nor do they convince one that the alterations in excitability of the nerves are strictly due to the withdrawal of calcium. From long previous experience, although it is known that a rise in excitability of a brief and temporary nature can come about from inadequacy of the circulation and that the great fall at the end of each curve is doubtless due to this or to some direct injury of other kinds, still it is also known that with a good circulation with normal blood the excitability will remain almost constant. These curves were especially inconclusive in that while a visible contraction could be elicited with weak currents, strong shocks gave only about the same muscular jerk. Instead of a brisk sharp contraction the foot moved lazily, often twisting in a peculiar way, and after the perfusion with dialyzed blood had gone on for only a short time all peripheral contractions disappeared and the shock produced only jerking of the thigh muscles behind the electrode. These are inevitable signs of approaching death of the nerve, impressing us rather as the consequences of some sort of poisoning, and in every case the dialyzed blood was quickly exchanged for normal blood, sometimes with a return of the excitability.

Discouraged by these results, we determined to add the normal amount of calcium to our dialyzing fluid, which at this time had the following composition:

Sodium chloride	60.0
Disodium phosphate	28.5
Potassium chloride	4.0
Magnesium chloride	2.87
Sodium bicarbonate	5.0
Dextrose	10.9
Water	10,000

It became apparent at once, as we might have foreseen, that the calcium is precipitated by the phosphates present. Phosphates were therefore left out and calcium was added. After dialysis with this fluid, perfusion of the leg gave almost the curve which one would expect with normal blood.



TEXT-FIG. 2. Perfusion of leg with blood dialyzed against fluid containing all inorganic constituents of blood including calcium.

Dog 1434.—(Text-figure 2.) Normal leg perfused with blood dialyzed against a fluid containing calcium but no phosphates.

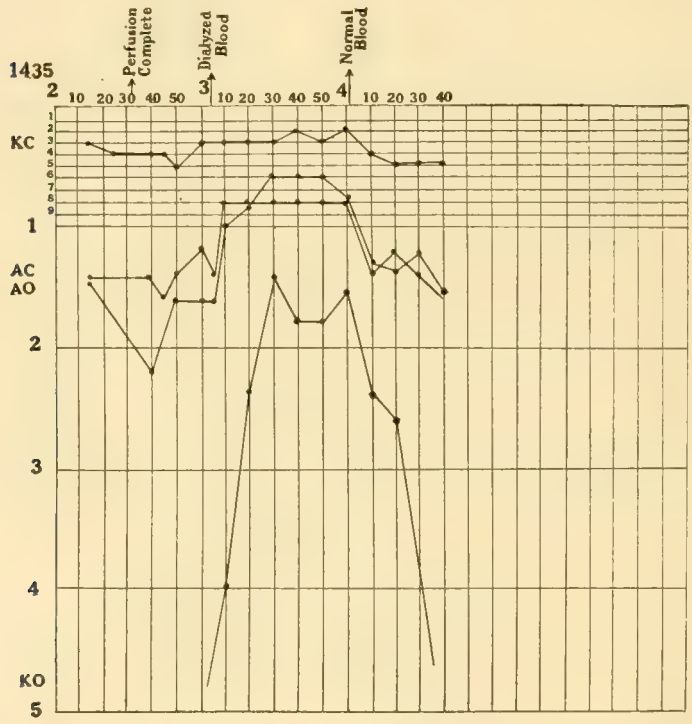
Sodium chloride	60.0
Sodium bicarbonate	5.0
Potassium chloride	4.0
Magnesium chloride	2.3
Dextrose	11.0
Calcium lactate	6.0
Water	10,000

	3.10	3.25	3.35	3.45	3.55	4.00	4.10	4.20	4.30	4.40	4.50	5.00	5.10	5.20
KC .	0.2	Perfusion	0.2	0.3	0.5	0.5	0.3	0.3	0.4	0.4	0.5	0.5	0.6	1.0
KO .	—		2.8	—	—	—	5.0	3.8	4.0	3.8	3.4	3.8	4.6	6.0
AC .	1.0		0.8	1.0	1.2	1.2	1.2	1.2	1.2	1.4	1.2	1.6	1.6	2.0
AO .	1.4		0.4	1.4	1.4	1.6	1.2	1.0	0.8	1.0	1.2	1.6	1.2	2.0

It will be seen from text-figure 2 that during the perfusion of normal blood a partial clamping off of the supply tube produced a

brief rise in excitability which then passed away when the current was turned on in its full strength.

Therefore, from that point the experiments were begun again with a dialyzing fluid containing neither calcium nor any considerable quantity of the disturbing phosphates. Probably with this fluid the blood lost some of its phosphates, but the difficulty of devising a dialyzing fluid which should remove nothing but calcium from the blood seemed insurmountable.[5]



TEXT-FIG. 3. Perfusion of leg with blood dialyzed against fluid devoid of calcium.

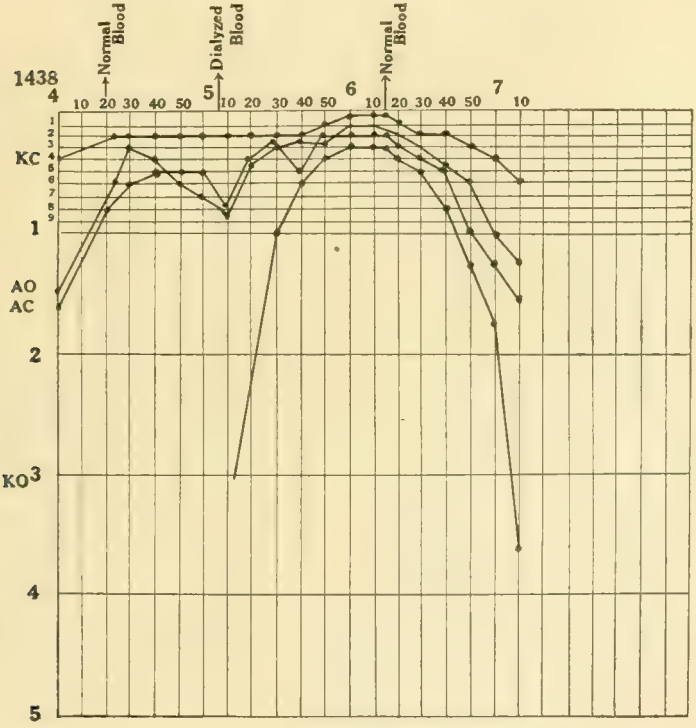
Dog 1435.—(Text-figure 3.) Normal leg perfused at 2.32 P. M. with normal blood, at 3.01 with dialyzed blood, and at 4.01 again with normal blood. 900 c.c. of normal blood were dialyzed for 7½ hours against 18,000 c.c. of fluid (fluid 3) of the following composition, which was used in all later experiments.

[5] Since writing this paper it has been suggested to us by Dr. A. E. Taylor that as the phosphates in the blood are in non-dialyzable combinations there is little source of error in using a dialyzing fluid without phosphates.

Sodium chloride	60.0
Sodium bicarbonate	5.0
Magnesium chloride	2.30
Potassium chloride	4.0
Dextrose	10.9
Water	10,000

	2.15	2.18	2.32	2.40	2.45	2.50	3.00	3.05	3.10	3.20	3.30	3.40	3.50	4.00	4.10	4.20	4.30	4.40
KC	0.3	0.4	Perfusion complete	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.4	0.5	0.5	0.5
KO	—	—		—	—	—	—	—	4.0	2.4	1.4	1.8	1.8	1.6	2.4	2.6	—	4.0
AC	1.1	0.8		1.4	1.6	1.4	1.2	1.4	0.8	0.8	0.8	0.8	0.8	0.8	1.4	1.2	1.4	1.6
AO	1.4	1.0		2.2	2.0	1.6	1.6	1.6	1.0	0.8	0.6	0.6	0.6	0.8	1.4	1.4	1.2	1.6

Dialyzed blood — Normal blood



TEXT-FIG. 4. Perfusion of leg with blood dialyzed against fluid devoid of calcium.

Dog 1438.—(Text-figure 4.) Normal leg perfused at 4.20 P. M. with normal blood. This was changed to dialyzed blood at 5.02, and again to normal blood at 6.12. The dialyzed blood was 1,000 c.c. which had been poured through the long celloidin tubules for 7 hours against 24,000 c.c. of the fluid described above.

	4.00	4.20	4.25	4.30	4.40	4.50	5.00	5.05	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.40	6.50	7.00	7.10
KC..	0.4	Perfusion complete	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.05	0.05	0.1	0.2	0.2	0.3	0.4	0.6
KO..	—		..	—	—	—	—	—	—	—	1.0	0.6	0.4	0.5	0.3	0.4	0.5	0.8	1.2	1.8	3.6
AC..	1.4		0.4	0.6	0.5	0.5	0.5	0.8	0.8	0.4	0.2	0.5	0.2	0.2	0.2	0.3	0.4	0.5	1.0	1.2	1.4
AO..	1.4		0.6	0.3	0.4	0.6	0.7	0.8	0.8	0.4	0.2	0.2	0.2	0.1	0.1	0.2	0.3	0.3	0.6	1.0	1.2

Normal blood — Dialyzed blood — Normal blood

On the entrance of the dialyzed blood the leg jerked and moved itself about. Twitchings were evident until about 5.20.

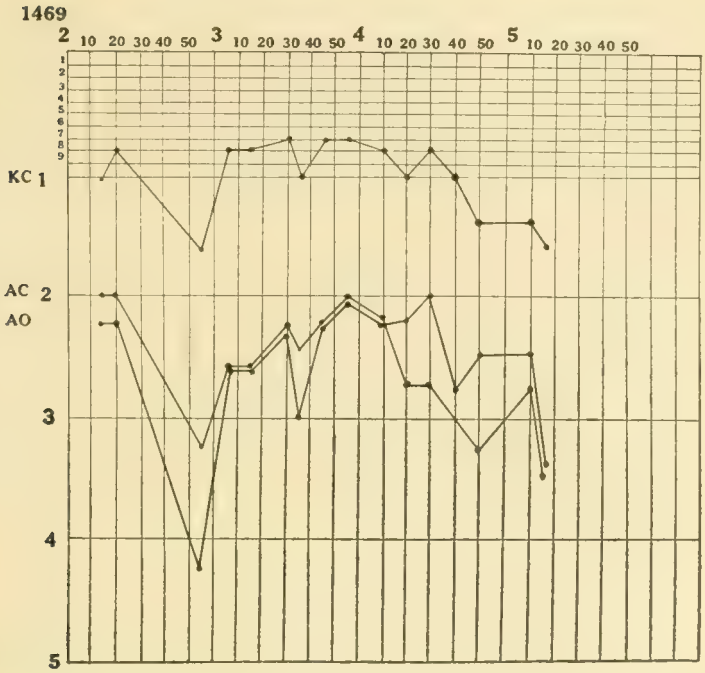
The chemical analysis of the dialyzing fluid before and after dialysis, and of the blood at various points in the experiments, is as follows. The figures show the amount of calcium per 1,000 c.c.

	Dog 1,435.	Dog 1,438.
Dialyzing fluid before dialysis of blood	0.0040	0.0043
Dialyzing fluid after dialysis of blood (1st)	0.0076	0.0076
Dialyzing fluid after dialysis of blood (2d)	0.0048	0.0054
Blood before dialysis	0.0364	0.0442
Blood after dialysis	0.0284	0.0233
Blood after perfusion of leg	0.0212(?)	0.0300

Text-figures 3 and 4 show clearly that perfusion with blood partly deprived of its calcium by dialysis produces great hyperexcitability of the nerves, and further that this must be due solely to the loss of calcium, since exactly the same procedure, changed only by the addition of precisely enough calcium to the dialyzing fluid to prevent any loss or gain of calcium by the blood, brings about no such hyperexcitability of the nerves. This proof may be somewhat strengthened by the addition of another curve made in the same way, except that the dialyzing fluid was so devised as to remove a large part of the chlorides from the blood, leaving the calcium unchanged.

Dog 1,469.—(Text-figure 5.) Normal leg perfused at 2.45 P. M. with normal blood, and at 3.30 with dialyzed blood. Dialysis was of 800 c.c. of normal blood for 6½ hours against 30,000 c.c. of fluid 4 of the following composition:

Sodium acetate	50.0
Sodium nitrate	40.0
Sodium bicarbonate	10.0
Magnesium chloride	2.3
Potassium nitrate	5.25
Calcium lactate	5.0
Dextrose	20.0
Water	10,000



TEXT-FIG. 5. Leg perfused with blood dialyzed against a chloride-free fluid.

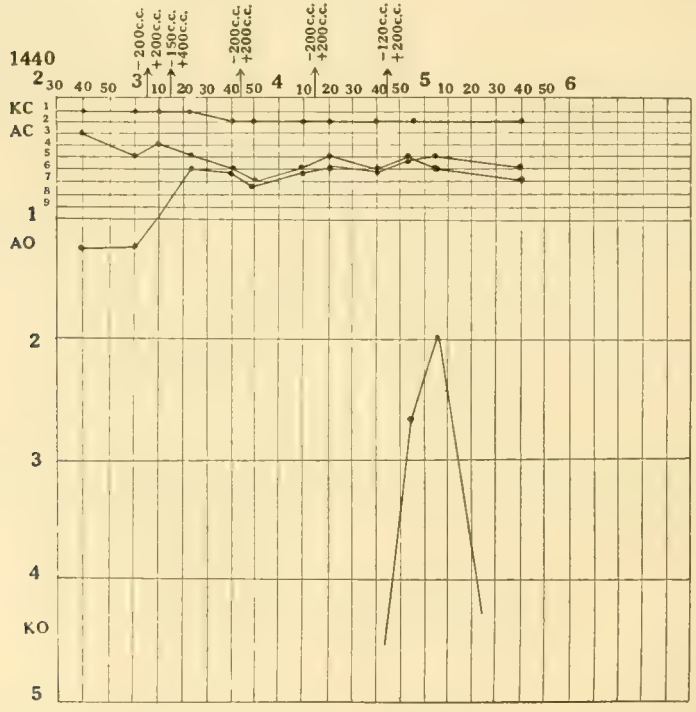
	2.15	2.20	2.45		2.55	3.05	3.15	3.30	3.35	3.43	3.55	4.10	4.20	4.30	4.40	4.50	5.10	5.15
KC...	1.0	0.8	Perfusion	Normal blood	1.6	0.8	0.8	0.7	1.0	0.7	0.7	0.8	1.0	0.8	1.0	1.4	1.4	1.6
KO...	—	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—
AC...	2.0	2.0			3.2	2.6	2.6	2.2	2.4	2.2	2.0	2.2	2.8	2.8	3.0	3.2	2.8	3.4
AO...	2.2	2.2			4.2	2.6	2.6	2.2	3.0	2.2	2.0	2.2	2.2	2.0	2.8	2.6	2.6	3.4

Dialyzed blood

It is seen from text-figure 5, in which the variations are so slight, that hyperexcitability of the nerves is not produced by long perfusion, even with blood greatly altered by dialysis, so long as the dialysis has not removed the calcium. When the calcium is diminished, however, the effect in producing hyperexcitability is prompt and persists as in the case of tetany blood so long as blood altered in this way is passing, giving way to normal conditions as soon as normal blood is again circulated.

When an isolated extremity is perfused the influence of the undiluted dialyzed blood must be felt by the nerves, since there is but little tissue from which calcium or other constituents could be

absorbed in the passage of the blood. We thought, therefore, that this might be the most trustworthy expression of the nature of the influence of the dialyzed blood. Nevertheless we attempted to introduce dialyzed blood in quantity into the veins of an animal, realizing that it would at once meet with an abundant source of calcium in the tissues, but hoping to circulate it so rapidly as to surprise the nervous system and produce some hyperexcitability not only of the peripheral nerves but of the ganglion cells. We planned not only to wash out all the animal's blood with dialyzed blood but to replace that, too, with fresh dialyzed blood. Indeed after one or two experiments we even circulated fresh dialyzed blood through the whole animal, so that if the tissues were to supply calcium to all the new blood presented to them they must furnish enough to bring back to normal the calcium content of five or six times as much blood as was possessed by the animal before. At the beginning of each



Text-Fig. 6. Repeated bleeding from carotid with replacement of blood dialyzed until poor in calcium

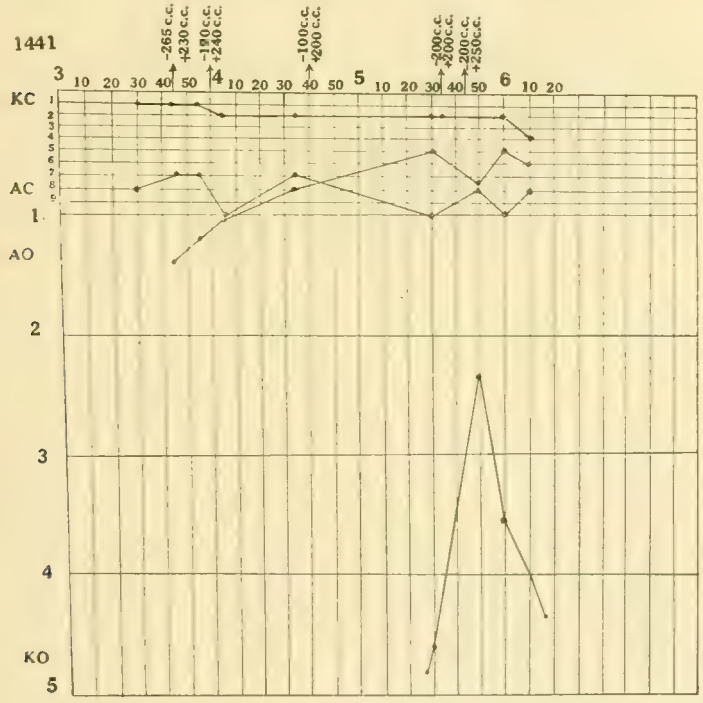
experiment the parathyroids of the animal were removed. The protocols are as follows:

Dog 1,440.—(Text-figure 6.) Normal animal bled at intervals, dialyzed blood being returned. Thyroids and parathyroids removed at 4.35 P. M. No tetany developed.

	2.40	3.00	3.10	3.25	3.40	3.50	4.10	4.20	4.45	4.55	5.05	5.40
KC.	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
KO.	—	—	—	—	—	—	—	—	—	2.6	2.0	—
AC.	0.3	0.5	0.4	0.5	0.6	0.7	0.6	0.5	0.6	0.5	0.6	0.7
AO.	1.2	1.2	1.0	0.6	0.6	0.7	0.6	0.6	0.6	0.5	0.5	0.6

Bled	200 c.c.	150 c.c.	200 c.c.	120 c.c.
Returned dialyzed blood	200 c.c.	400 c.c.	200 c.c.	200 c.c.

In this experiment 1,350 c.c. of blood dialyzed for 7 hours against 30,000 c.c. of fluid 3 were used in replacing normal blood. Effect very slight.



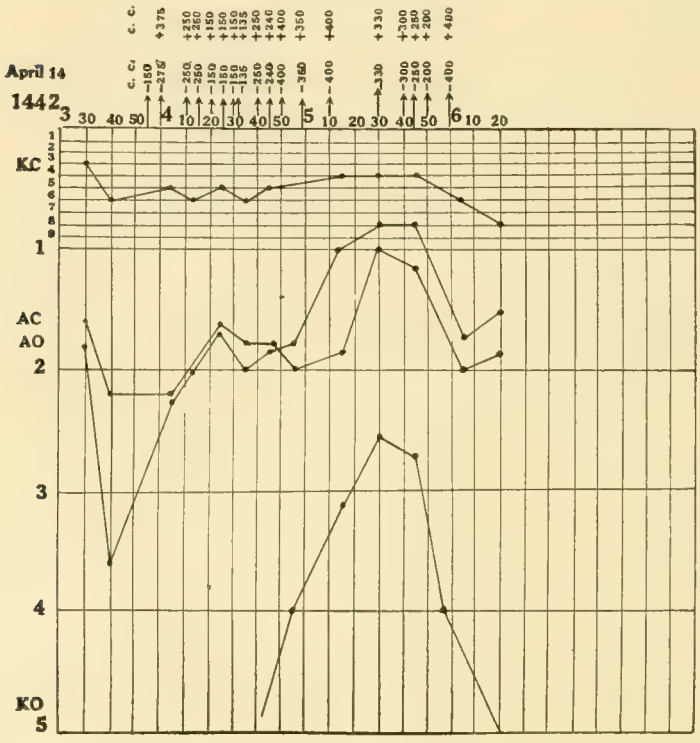
TEXT-FIG. 7. Normal animal. Blood replaced at intervals with dialyzed blood.

Dog 1,441.—(Text-figure 7.) Normal animal, blood at intervals replaced with dialyzed blood. 1,300 c.c. of blood dialyzed for 7½ hours against 26.000 c.c. of fluid 3. Parathyroids were removed just before the experiment started.

	3.30	3.45	3.55	4.05	4.35	5.30	5.50	6.00	6.10
KC .	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.4
KO .	—	—	—	—	—	4.6	2.4	3.6	4.0
AC .	0.8	0.7	0.7	1.0	0.7	1.0	0.8	1.0	0.8
AO .	1.4	1.4	1.2	1.0	0.8	0.5	0.8	0.5	0.6

Bled	265 c.c.	190 c.c.	200 c.c.	200 c.c.
Returned dialyzed blood	230 c.c.	240 c.c.	200 c.c.	250 c.c.

There is general twitching; no definite tetany; much vomiting.



TEXT-FIG. 8. Normal animal through which dialyzed blood was actually circulated.

Dog 1,442.—(Text-figure 8.) Normal animal through whose blood vessels dialyzed blood was actually circulated, as shown in the chart. Parathyroids first removed.

	3.20	3.40	4.05	4.12	4.25	4.35	4.45	4.55	5.15	5.30	5.45	6.05	6.20
KC...	0.3	0.6	0.5	0.6	0.5	0.6	0.5	0.5	0.4	0.4	0.4	0.6	0.8
KO...	—	—	—	—	—	—	—	4.0	3.2	2.6	2.8	4.0	5.0
AC...	1.6	2.2	2.2	2.0	1.6	1.8	1.8	2.0	1.8	1.0	1.2	2.0	1.8
AO...	1.8	3.6	2.2	2.0	1.6	2.0	1.8	1.8	1.0	0.8	0.8	1.8	1.6

Bled	150 c.c.	275 c.c.	250 c.c.	250 c.c.	150 c.c.	150 c.c.	135 c.c.	250 c.c.	240 c.c.	400 c.c.	350 c.c.	400 c.c.	330 c.c.	300 c.c.	250 c.c.	200 c.c.	400 c.c.
Returned dialyzed blood	375 c.c.		250 c.c.	250 c.c.	150 c.c.	150 c.c.	135 c.c.	250 c.c.	240 c.c.	400 c.c.	350 c.c.	400 c.c.	330 c.c.	200 c.c.	250 c.c.	200 c.c.	400 c.c.

1,800 c.c. of blood dialyzed for 7 hours against 30,000 c.c. of fluid 3 were used. Each part of this probably circulated two and one half times. Vomiting; no definite twitching; collapse and death.

From these experiments it seems clear that it is impossible in a short time to reduce the calcium content of the blood circulating through the whole body or to keep it reduced to such a degree as to bring about a tetany-like hyperexcitability of the nerves. There is too abundant a source for fresh calcium in the various tissues, including the bones, and the blood quickly replenishes itself. It might seem possible to repeat this process during several days and thus imitate the latent period which follows extirpation of the parathyroids, but in this case it would be necessary to extirpate those glands, when tetany would arise of itself, since otherwise the blood would rapidly replenish its calcium, under their influence, from the skeleton. Indeed, the rapidity with which calcium enters the circulating dialyzed blood from the tissues is shown by comparison of the analyses of samples taken before and after its circulation in the body.

In dog 1,441 the blood contained per 1,000 c.c.:

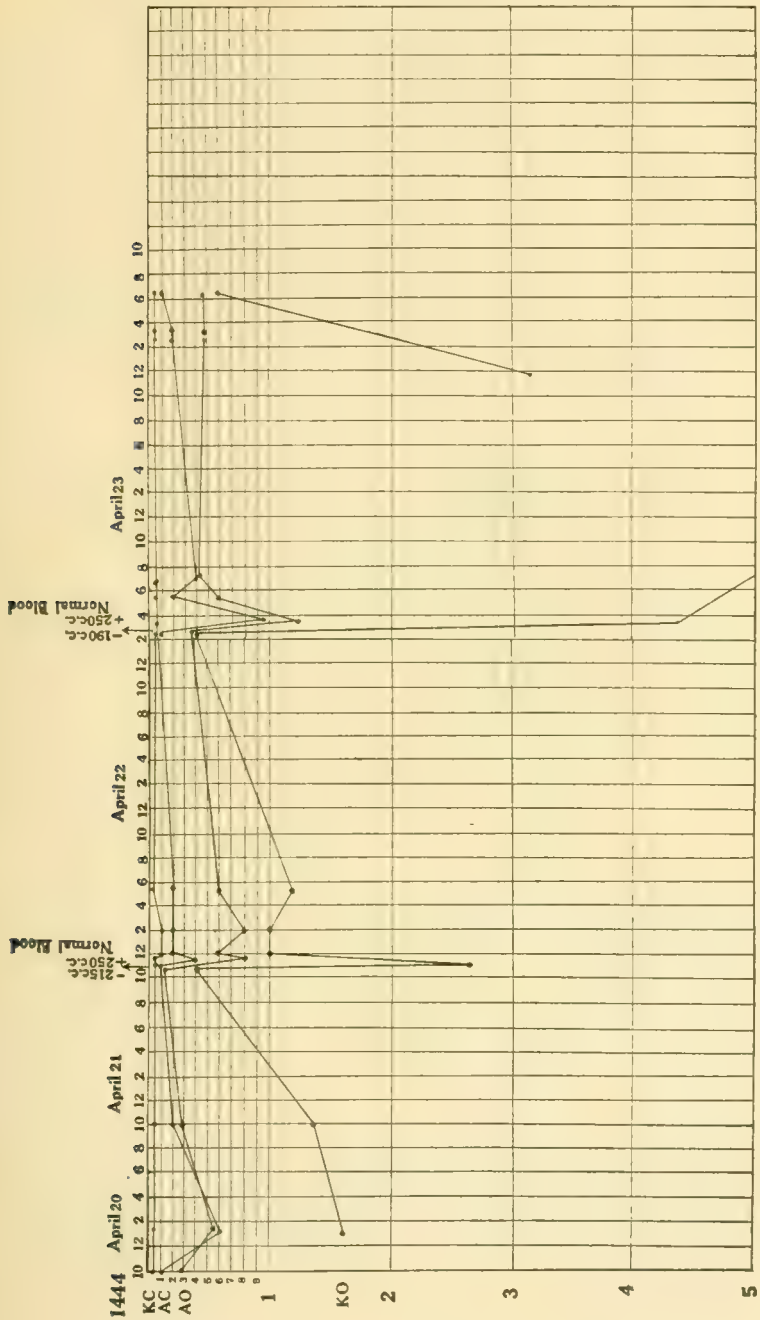
Before dialysis	0.0485
After dialysis	0.0190
After circulation for about 2 hours	0.0381

It remains a question whether, if one could continue this depletion over several days, it would be possible to produce such changes in excitability as are seen in tetany, and in fact this was our original problem. It leaves one with the impression that the parathyroid secretion must govern the conversion of a non-dialyzable and useless combination of calcium into a dialyzable form which is essential for the control of the excitability of the nerves, but which is lost

in the excreta and not newly formed in the absence of parathyroid secretion.

Although we had learned that it is possible by perfusion with calcium-poor blood to produce hyperexcitability of the nerve, it remained to bring this fact into direct relation with the condition in tetany, for it might be objected that there the mechanism is a different one. This we thought would be attained if we could show that this dialyzed blood had lost the power possessed by normal blood of curing tetany on transfusion. It is well known that if an animal in tetany, after the removal of the parathyroids, be bled and the blood replaced with normal blood, the excitability of the nerves falls, and twitchings disappear for a time. Dialyzed blood poor in calcium has little or nothing of this effect, although if the dialyzed blood be not so poor in calcium as that of the animal in tetany, it will lower the excitability somewhat. If in an animal in tetany we replace the blood with dialyzed blood and find no change in the symptoms nor lowering of the excitability, we may remove that blood and replace it with normal blood which does stop the twitching and lower the excitability.

But if, although the excitability is not lowered by the dose of dialyzed blood, we do nothing further, the excitability is found to be lowered the next day or after some hours. To explain this we have imagined that while in dialysis we remove the calcium from the normal blood, we may not remove the parathyroid secretion which on being injected into a dog in tetany after a time causes the appearance of dialyzable calcium in the blood and goes far to relieve temporarily the tetany. It is on this basis that it seems hopeless to attempt to produce a condition resembling tetany by the repeated replacement of the blood over a period of days with normal blood dialyzed until it is poor in calcium, because at the same time we furnish parathyroid secretion which ensures the continual withdrawal of calcium from the tissues. It is true that we might dialyze tetany blood, but even then having a blood free from parathyroid secretion and poor in calcium we have no animal in which to make evident the effect of pure lack of calcium, for the animal with intact parathyroids will continually frustrate this object, and the animal deprived of parathyroids will develop tetany during the experiment.

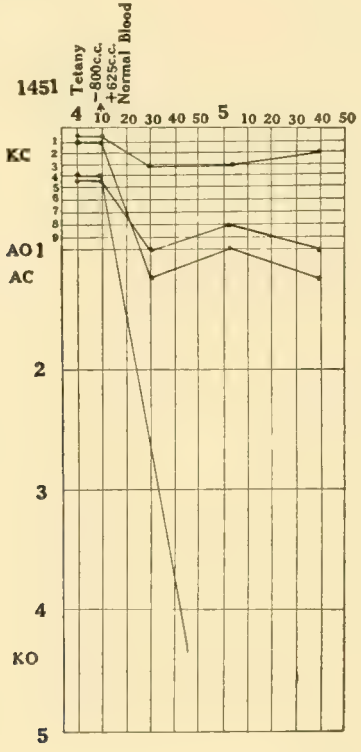


TEXT-FIG. 6. Tetany. Bled on two occasions; normal blood replaced. Transient effect.

ILLUSTRATIVE PROTOCOLS.

Dog 1,444.—(Text-figure 9.) Tetany developing 2 days after parathyroidectomy. Bled on two occasions, normal blood being replaced. Transient relaxation and lowering of excitability.

	April 20.		April 21.						April 22.				April 23.		
	10.00	1.45	10.00	11.15	11.40	12.00	2.00	5.45	2.30	2.55	5.30	6.35	2.20	3.10	6.30
KC	0.05	0.05	0.05	0.05	0.05	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO	.....	1.6	1.4	0.4	2.6	1.0	1.0	1.2	0.4	4.4	—	—	—	—	0.6
AC	0.1	0.6	0.2	0.05	0.4	0.2	0.2	0.2	0.05	1.0	0.2	0.4	0.2	0.2	0.1
AO	0.3	0.6	0.3	0.1	0.8	0.6	0.8	0.6	0.4	1.2	0.6	0.4	0.5	0.5	0.5
Bled					215 c.c.				190 c.c.						
Returned normal blood					250 c.c.				250 c.c.						



TEXT-FIG. 10. Tetany. Effect of bleeding and replacing normal blood.

Dog 1,451.—(Text-figure 10.) Violent tetany 24 hours after parathyroidectomy. Bled 800 c.c., and this loss was replaced by 625 c.c. of normal blood. Immediate relaxation and lowering of excitability of nerves.[6]

[6] A curious phenomenon was observed in these cases in which nearly all the

	April 26.					April 27.	April 28.
	4.00	5.00	5.20	5.55	6.30	11.00	12.15
KC.....	0.05	Blood replaced by normal	0.3	0.3	0.2	0.1	0.05
KO.....	0.4		—	—	—	—	0.3
AC.....	0.1		1.2	1.0	1.2	1.2	0.05
AO.....	0.4		1.0	0.8	1.0	1.2	0.2

Bled 800 c.c. 425 c.c.
Returned normal blood 625 c.c. Returned dialyzed blood 400 c.c.

This animal developed tetany again on April 28 and was bled 425 c.c., 400 c.c. of dialyzed blood being replaced. There was no recovery from the spasm brought on by the bleeding, and death followed rapidly. This case forms an example of the contrast between the effects of introducing normal and dialyzed blood.

Dog 1,445.—Moderate tetany 2 days after parathyroidectomy. At 2.23 P. M. bled 150 c.c. Blood replaced by 150 c.c. of blood dialyzed for 5 hours against 10,000 c.c. of fluid 3. At 3.20 bled 150 c.c., and replaced 150 c.c. of normal blood. At 10.30 bled 200 c.c., and replaced 250 c.c. of normal blood.

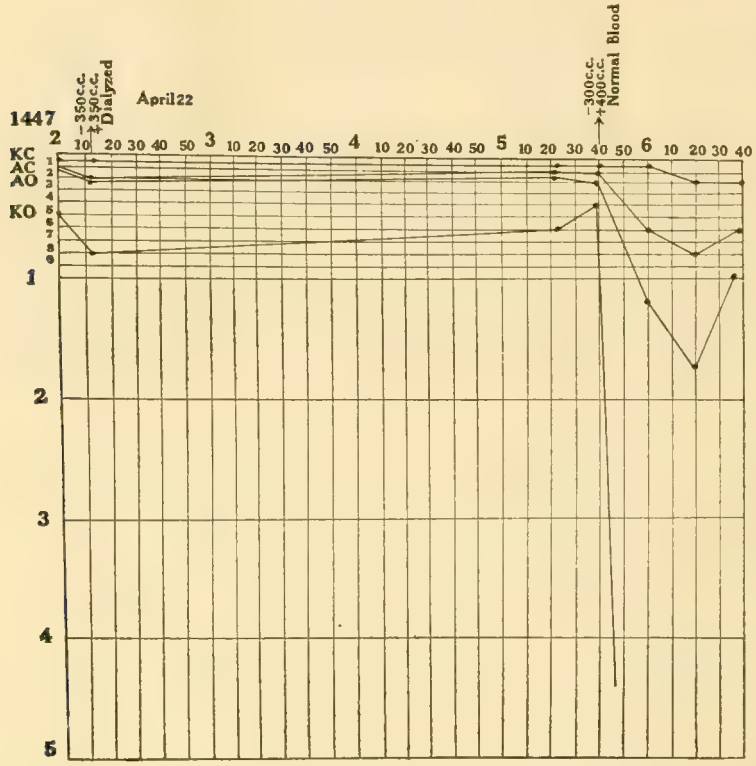
	April 20.								April 21.							
	1.30	1.45	2.20	2.30	2.40	3.00	3.20	3.45	4.25	10.00	10.45	11.00	11.15	12.00	2.00	5.45
KC.	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO.	0.2	0.3	0.3	0.3	0.4	0.3	0.6	1.4	0.6	0.2	0.4	0.4	0.4	—[7]	2.0	1.4
AC.	0.05	0.1	0.05	0.05	0.05	0.05	0.1	0.2	0.1	0.05	0.05	0.1	0.1	0.1	0.2	0.3
AO.	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.5	0.3	0.3	0.4	0.5	0.4	0.4	0.3	0.3

Bled 150 c.c. 150 c.c. 200 c.c.
Returned dialyzed blood 150 c.c. Returned normal blood 150 c.c. 200 c.c.

Dog 1,447.—(Text-figure 11). Violent tetany at 2 P. M. Bled 350 c.c. which were replaced with 350 c.c. of blood from a lot of 600 c.c. which had been dialyzed for 4½ hours against 12,000 c.c. of fluid 3. Change in character of electric reaction, but no marked change in excitability. Contraction slow, no rigidity. Tongue tremor and twitchings continue. At 5.45 bled 300 c.c., and replaced 400 c.c. of normal blood. Complete relaxation and return of excitability to normal.

blood was removed, at the moment before the normal blood was allowed to run in. Although twitching may have been slight when the bleeding began, the most violent contractions and extreme rigidity appeared during the extreme anemia, to disappear at once when the other blood entered the veins. Probably this is due to temporary asphyxia of the nerves.

[7] Negative up to 3 milliamperes.



TEXT-FIG. 11. Tetany. Bleeding on two occasions. Dialyzed blood replaced without effect. Normal blood reduces excitability.

	April 22.										
	11.45	2.00	2.15	2.55	5.25	5.40	6.00	6.20	6.45	9.00	12.00
KC..	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.2	0.2	Quiet;	Twitch-
KO..	0.5	0.5	0.8	Tongue and muscle tremor	0.6	0.4	—	—	—	no tongue tremor	ing; tremor in tongue.
AC..	0.1	0.1	0.2		0.1	0.1	0.6	0.8	0.6		
AO..	0.1	0.1	0.2		0.1	0.2	1.2	1.8	1.0		
Bled			350 c.c.				300 c.c.				
Returned dialyzed blood			350 c.c.		Returned normal blood		400 c.c.				

Dog 1,452.—No tetany 4 days after parathyroidectomy. Moderately high excitability. Bled 315 c.c., replaced by 330 c.c. of blood dialyzed 7 hours against fluid 3, $\frac{430 \text{ c.c. blood}}{10{,}000 \text{ c.c. fluid}}$. Animal much depressed and vomited. Excitability a

little lowered, and this lowering continued and increased for the next two days. Then bled 300 c.c., which were replaced by 300 c.c. of blood dialyzed for 6 hours, $\frac{400 \text{ c.c.}}{15{,}000 \text{ c.c.}}$. No marked change in excitability.

	April 29.					May 1.				
	5.00	5.25	5.35	5.50	6.05	2.00	5.30	5.40	5.50	6.25
KC.....	0.05	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1
KO.....	1.8	2.6	2.0	2.0	2.0	—	—	—	—	—
AC.....	0.4	0.8	0.8	1.2	1.0	1.4	1.2	1.2	1.2	1.2
AO.....	0.5	0.8	0.8	1.0	0.8	2.0	1.4	1.4	1.6	2.0
Bled		315 c.c.					300 c.c.			
Returned dialyzed blood		330 c.c.					300 c.c.			

We made a number of analyses of the blood of animals in tetany, of the dialyzed normal blood used to replace their own, of the normal blood, and of the normal or dialyzed blood removed again after it had circulated for a time in the animal, thinking that a comparison of these figures might throw some light on what occurred in the body to influence the tetany. But such figures would be difficult to interpret because they would change continually with the time the new blood was allowed to stay in the body, first through the process of obtaining a balance with the tissues, and then through the same process that disturbed the composition of the blood to produce tetany. It might be expected then that thoroughly dialyzed blood left in a tetany animal would become richer in calcium for awhile, although normal blood introduced in the same way would steadily lose calcium until it became very poor indeed, when another attack of tetany might come on, as in dog 1,451.

If tetany blood be dialyzed under exactly the same conditions as normal blood, it still loses a proportionate amount of its calcium, which would perhaps show that it is not especially the loss of a diffusible calcium as contrasted with a non-diffusible form which is important in producing tetany. The blood of dog 1,451 taken during tetany contained 0.0287 of a gram of calcium per 1,000 cubic centimeters. 500 cubic centimeters dialyzed for 5 hours against 12,000 cubic centimeters of fluid 3 then contained 0.0114 of a gram per 1,000 cubic centimeters.

CONCLUSIONS.

We may devise a fluid containing practically all the inorganic diffusible constituents of the blood except calcium, and use it to dialyze normal blood in such a way as to remove from it a large part of its calcium. The dialyzed blood when perfused through an isolated extremity produces an extreme hyperexcitability of the nerves quite like that observed in tetany. Since perfusion with blood dialyzed in precisely the same way against a fluid of the same composition, but containing calcium in the proportion found in the normal blood, causes no hyperexcitability of the nerves, it is evident that the hyperexcitability is due to the lack of calcium. This effect can be attained in only a slight degree by replacing the blood of a whole animal with the dialyzed blood, since under the conditions of the experiment the tissues cannot be sufficiently depleted of their calcium. It seems probable that the parathyroid secretion is not removed by dialysis, but is returned to the body with the dialyzed blood.

To bring this result into relation with the condition in tetany following parathyroidectomy, animals in tetany were bled and the blood was replaced in one case with normal blood, in the other with dialyzed blood poor in calcium. The normal blood immediately relieves the tetany and lowers the excitability, while the dialyzed blood does not. We therefore believe that this is a further proof that in the tetany of parathyroidectomy also the twitching and hyperexcitability of the nerves is due to lack of calcium in the blood and tissues.

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THE RÔLE OF THE SPLEEN IN BLOOD FORMATION.*

BY DUDLEY H. MORRIS, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

Various authors[1] have described the appearance in the spleen in the course of chronic anemias of abundant cells which give evidence of the new formation of blood in that organ when blood formation is greatly needed by the body. These cells are erythroblasts and mononuclear cells of various sizes which become very abundant in the venous sinuses. The same cells are found in the capillaries of the liver and occasionally outside these, in the periportal tissues, but not in great numbers in the circulating blood.

The information derived from attempting to estimate the change in the numbers of cells in such an organ as the spleen was felt to be unsatisfactory and a method of study other than the staining of sections of the fixed spleen was sought. Without any knowledge of previous efforts in this direction it was thought that accurate counts of the blood leaving the spleen by the splenic vein when compared with similar counts of that entering by the splenic artery should give a clear idea of the activity of the spleen with respect to its contribution of cells to the blood. Since the completion of the work a few references to similar studies have been found, but it nevertheless seems that a detailed statement of the result of these counts would be of value, especially since the method which is so useful has been employed by hardly any modern workers.

* Received for publication, August 5, 1914.

[1] Bizzozero, G., and Salvioli, G., *Arch. p. le sc. med.*, 1880, iv, 49. Meyer, E., and Heineke, A., *Verhandl. d. deutsch. path. Gesellsch.*, 1906, ix, 224; *Deutsch. Arch. f. klin. Med.*, 1907, lxxxviii, 435. Morris, R. S., *Bull. Johns Hopkins Hosp.*, 1907, xviii, 200. von Domarus, A., *Arch. f. exper. Path. u. Pharmakol.*, 1908, lviii, 319. Heinz, R., *Virchows Arch. f. path. Anat.*, 1902, clxviii, 501. Jolly, J., and Rossello, H., *Compt. rend. Soc. de biol.*, 1909, lxvi, 40. Schridde, H., *Centralbl. f. allg. Path. u. path. Anat.*, 1908, xix, 865. Naegeli, O., Ehrlich, P., and Lazarus, A., Die Anaemie, Vienna and Leipzig, 1909.

It has been known for a long time that the leucocytic count of the blood from the splenic vein exceeds that of the artery.

Vierordt[2] in four counts made on an executed man found the relation of leucocytes to red corpuscles in the splenic vein to be 1 : 49. Hirt[3] found that in the calf the relation was 1 : 60, while in the arterial blood it is 1 : 2,200. Funke[4] gives this relation in the splenic vein as 1 : 4, while Frey[5] calculated the proportion in an old man dead of pneumonia as 1 : 102. Rindfleisch[6] called attention to the richness of the splenic blood in leucocytes especially after a meal. Weidenreich[7] undertook counts from the sinuses of the spleen and obtained variable results inasmuch as some of them contained few or no leucocytes, while others were filled with them doubtless because those sinuses into which the arterial stream enters directly will contain predominantly red corpuscles while the reverse condition will obtain where the lymph channels enter. As a mean I found a proportion of 1 : 15. If we take the relation in the artery (following Hirt) as 1 : 2,200, and the average of the figures from the literature as 1 : 30 it is seen that the splenic vein contains about seventy times as many leucocytes as the efferent artery. Since special efferent lymphatic channels for the drainage of the lymphocytes of the spleen have been shown not to exist, the cells must be discharged into the vena lienalis which thus contains both blood and lymph.

Von Ebner[8] states that it has been shown that the blood of the splenic vein contains an uncommonly large number of white corpuscles and that the site of their formation is the splenic tissue itself from which they enter through the permeable walls of the venules. Loewit[9] studied the relation of leucocytes of arterial blood and splenic venous blood in the guinea pig and found 30 to 80 times as many leucocytes in the venous blood, the increased proportion being due to small and large mononuclear elements. This finding is not obtained in all animals probably because of the irregular or intermittent discharge of the cells. Certain negative results of Tarchanoff and Swaen[10] are probably due to this cause.

The cardinal idea, therefore, in the present study was to examine carefully the blood entering the spleen and to compare it with that emerging by the splenic vein. Any differences should

[2] Vierordt, *Arch. f. physiol. Heilk.*, 1854, xiii, 259, 408.

[3] Hirt, E., *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1856, 174.

[4] Funke, O., Lehrbuch der Physiologie, Leipzig, 1863, i.

[5] Frey, H., Handbuch der Histologie und Histochemie des Menschen, 4th edition, Leipzig, 1874.

[6] Rindfleisch, G. E., Experimentelle Studien ueber die Histologie des Blutes, Leipzig, 1875.

[7] Weidenreich, F., *Arch. f. mikr. Anat.*, 1901, lviii, 247.

[8] von Ebner, V., *Köllikers Handbuch der Gewebelehre*, 1902, iii, 276.

[9] Loewit, M., *Folia Haematol.*, 1907, iv, 473.

[10] Tarchanoff, J., and Swaen, A., *Arch. de physiol. norm. et path.*, 1875, ii, series 2, 324.

represent the changes produced by the spleen. Counts were made with the Thoma-Zeiss apparatus with blood from the splenic artery, splenic vein, mesenteric vein, and, for purposes of control, from a peripheral systemic vein. Smears of blood were also made from each situation. Rabbits, cats, and dogs were studied, some in perfect health, others suffering from spontaneous or artificially induced disease. Thorough autopsies were made in each case and the organs studied microscopically. The counts were made in the usual way but with the most scrupulous attention to technical accuracy in every detail. For the white corpuscles 0.2 per cent. acetic acid was used and for the red cells fresh Toison's solution. Differential counts of the white cells were made in all cases for the blood from each situation.

In securing blood from the splenic artery and vein considerable care must be taken to have a dry field without great loss of blood in the earlier steps of the operation. An incision from the midline along the left costal margin to the axillary line was found to give the best exposure, after which the assistant could elevate the spleen without making traction upon the vessels, by [illegible] and gently drawing the spleen forward so as to expose the hilum. Care was taken not to handle or squeeze the spleen so as to force out its contents artificially. The remaining viscera were protected and the spleen supported by warm cloths so that the [illegible] ceeded normally. The blood must be obtained from the splenic vein first, as interference with the arterial current will produce changes in that of the veins, while the venous anastomosis and collateral trunks are so numerous that occlusion of one produces no effect.

Some counts were made from blood allowed to spurt from a puncture in the vein, but since this hemorrhage makes it difficult to work later with the artery it was found advisable to put on two Carrel clamps and make the puncture between them. The latter is the more satisfactory procedure since a few drops only may be allowed to flow, and the counts do not differ appreciably from those made from the freely flowing blood.

Ehrlich's triacid stain after fixation by heat and Jenner's, Wright's, and Giemsa's stains after methyl alcohol fixation were

used for the differential counts. Prolonged staining with diluted Giemsa's stain gave good results.

The experiments showed that the blood of the splenic vein differs remarkably from that of the artery not only in the character of its cells but in their number, and this difference was found to exist also between the blood of the splenic vein and that of a peripheral systemic vein.

These differences may be summed up as follows:

(1) The number of red corpuscles per cubic millimeter in the blood of the splenic vein is greater than in that of the artery.

(2) Similarly the number of white corpuscles in the venous blood is greater than in the arterial blood.

(3) The character and proportion of the several varieties of white corpuscles is different in the splenic vein from what is found in the artery. Large mononuclear leucocytes appear in great excess there.

(4) The blood of the splenic vein contains more perfect and larger red corpuscles than that of the artery and they seem richer in hemoglobin.

(5) The blood of the inferior mesenteric veins differs from that of the splenic vein in being relatively richer in small mononuclear cells and poorer in the large mononuclears.

In the tabulated and detailed results given below (table I) these differences are more definitely brought out. It will be noted that the counts made from the ear vein and splenic artery of the same animal correspond very closely, as would be expected, while a marked difference was always found when blood was taken from the splenic veins.

It will be seen from table I that when the autopsy showed the animals operated on to be normal the results were invariably the same. There were always more red and white cells in the splenic veins than could be found in any of the other vessels. Frequently this excess of corpuscles rose to such a degree that there were twice as many red or white cells coming out as going into the spleen. That the excess of white cells should be mainly of the mononuclear variety was to be expected. But there occurred a further noteworthy fact, namely, that these mononuclears were mainly of the

TABLE I.

Animal.	Blood count.	Ear vein.	Splenic artery.	Splenic vein.	Inferior mesenteric vein.	Autopsy.
Rabbit 1	Red blood count	5,400,000	5,200,000	6,700,000		Small nodule of coccidiosis in liver, otherwise normal.
	White blood count	4,000	4,000	9,400		
	Polynuclears	60%	61%	32%		
	Mononuclears	40%	39%	68%		
Rabbit 2	Red blood count	5,900,000	5,700,000	8,600,000		All organs appear normal.
	White blood count	10,600	10,000	25,600		
	Polynuclears	47%	49%	20%		
	Mononuclears	53%	51%	80%		
Rabbit 3	Red blood count	4,600,000	4,560,000	5,360,000		Young rabbit, normal.
	White blood count	5,200	5,000	1,600		
	Polynuclears	30 to 32%	30 to 32%	18 to 20%		
	Mononuclears	68 to 70%	68 to 70%	80 to 82%		
Rabbit 4	Red blood count	Not done	5,280,000	6,800,000		Lactating rabbit. Marked coccidiosis in liver. Spleen small, shrunken, and dark red.
	White blood count	9,800	9,400	4,600	Portal vein	
	Polynuclears	38%	36%	10%	32%	
	Mononuclears	62%	64%	90%	68%	
Dog 1	Red blood count	5,280,000	5,280,000	6,640,000		Normal.
	White blood count	17,000	16,400	34,400	16,400	
	Polynuclears	90%	85%	70%		
	Mononuclears	10%	15%	30%		
Dog 2	Red blood count	4,500,000	4,320,000	6,600,000		Normal.
	White blood count	6,900	7,000	14,800	10,600	
	Polynuclears	61%	60%	44%	45%	
	Mononuclears	39%	40%	56%	55%	
	Large mononuclears			47%	10%	
	Small mononuclears			9%	45%	
Dog 3	Red blood count	5,900,000	5,800,000	7,240,000		All organs normal.
	White blood count	19,700	19,700	19,900		
Dog 4	Red blood count	4,200,000	4,100,000	6,500,000		All organs normal.
	White blood count	20,000	20,000	25,000		
	Polynuclears	80%	81%	70%		
	Mononuclears	20%	19%	30%		
	Large mononuclears	12%	12%	24%		
Cat 1	Red blood count		4,400,000	9,120,000		Apparently normal.
	White blood count		18,000	44,000		
	Polynuclears		75%	30%		
	Mononuclears		25%	70%		

TABLE I.—*Continued.*

Animal.	Blood count.	Ear vein.	Splenic artery.	Splenic vein.	Inferior mesenteric vein.	Autopsy.
Cat 2	Red blood count		9,600,000	13,104,000	9,440,000	All organs apparently normal except spleen which is enlarged to about 4 times its normal size and is very mottled on cross-section, due to tremendously enlarged Malpighian bodies. Two accessory spleens presenting same structure. *Microscopical Examination.*—Enormously enlarged follicles. Active mitoses. Huge mononuclear cells.
	White blood count		89,400	137,200	89,400	
	Polynuclears		75%	73%	75%	
	Mononuclears		25%	26%	25%	
	Large mononuclears		25%	20%	20%	
	Small mononuclears		0	1%	5%	
Cat 3	Red blood count		7,280,000	11,200,000		Spleen appears normal in size, color, shape, and cross-section. Liver normal. All other organs except mesenteric lymph nodes are normal. Mesenteric lymph nodes especially in duodenal region are enormously enlarged, firm, and matted together in places. Dark red in color. *Microscopical Examination.*—Sections of lymph nodes show tremendous enlargement of germinal centers. Long rod-like bacilli in marginal sinuses. Sections of spleen and liver normal.
	White blood count		26,000	13,200	26,000	
	Polynuclears		18%	40%	19%	
	Mononuclears		82%	60%	81%	
	Large mononuclears		11%	50%	9%	
	Small mononuclears		71%	10%	72%	

TABLE I.—*Concluded.*

Animal.	Blood count.	Left ventricle.	Right auricle.	Splenic vein.	Portal vein.	Autopsy.
Received intraperitoneal inoculation of virulent paratyphoid culture 24 hrs. previously.						
Rabbit 5	Red blood count	4,300,000		6,240,000		Peritonitis. Focal necroses in liver. Large mitotic cells in spleen, especially at margins of follicles.
	White blood count	[illegible]		7,500		
	Polynuclears		40%	[illegible]	88%	
	Mononuclears		[illegible]	88%	[illegible]	

large variety, whereas those coming from the adenoid tissue of the intestines and mesenteric nodes were mainly of the small variety. The significance of this was clearly shown in cat 3, where the white cells coming from the spleen were found to be only half as numerous as in the peripheral circulation and in the inferior mesenteric vein. The autopsy showed the reason for this. The spleen was unchanged and discharged its normal quota of large mononuclears into the blood stream; but the adenoid tissue in the mesentery was enormously hypertrophied and was discharging great numbers of small lymphocytes into the circulation. Microscopic sections showed the efferent vessels of these nodes to be packed with small mononuclears.

One of the most striking evidences of splenic blood formation was that of cat 2, in which 13,000,000 red cells and 137,000 white cells per cubic millimeter were found in the splenic vein as against about two thirds of that number in the peripheral circulation. The reason for this at once became apparent on examination of the spleen itself. There was an enormous hyperplasia of the organ, and the germinal centers on microscopical section showed very active production of large mononuclears. There were furthermore two accessory spleens. The organs elsewhere were normal and the cat seemed in good health.

Another marked instance of the difference between splenic and portal corpuscular content was seen in dog 2, in which the proportions of large and small mononuclears were almost exactly the reverse in the two veins.

Most interesting were the results in rabbit 5, which had been previously inoculated with a rather virulent strain of paratyphoid

bacillus. This strain in rabbits causes a marked leucopenia in the systemic vessels as a constant feature following inoculation. But the remarkable thing was that although this leucopenia had reached such a grade that the heart's blood contained only 450 white blood cells to the cubic centimeter the spleen still kept discharging leucocytes at a rate of 7,500 per cubic centimeter, a perfectly evident effort at compensation for the loss in the general blood stream.

These results all point to the inevitable conclusion that the spleen is a blood-forming organ of prime importance in the animal metabolism. The fact that the organ can be extirpated without causing death or even considerable detriment to the animal organism does not militate against this conclusion. Other organs (hemolymph nodes, bone marrow, and adenoid tissues in general) may assume part of the rôle of the spleen when this is absent, but only the severity of the blood-destroying agent and the individual resistance can determine whether the body can stand the strain when deprived of the spleen. Cases of death from removal of the malarial spleen indicate this strongly. And this same individual variation will doubtless account for the discrepancy in the results obtained by different observers regarding the importance of the spleen in the animal economy.

In closing, I desire to express my gratitude to Dr. William G. MacCallum for his interest in this work.

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THE RELATION OF BACTERIOLYSIS TO PROTEOLYSIS.

Studies on Ferment Action. XVI.*

By JAMES W. JOBLING, M.D., and WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The relation of the proteolytic ferments of serum to immunity reactions, particularly to bacteriolysis, has interested several investigators, and in view of the recent work of Abderhalden and his collaborators it merits attention quite apart from a theoretical consideration. The study of possible proteolytic cleavage during bacteriolysis, has, because of the manifest technical difficulties entailed by the accurate determination of nitrogen in the small amounts available, been limited to rather general statements, based, in most instances, on experiments carried out with the Sörensen method. This method, being an index of the total cleavage of the protein molecule, gives no information of the change from coagulable to non-coagulable forms, and is therefore of use in estimating peptolytic or ereptic enzyme activity rather than proteolytic changes. For a like reason it represents the splitting of the protein bodies to non-toxic fragments in contradistinction to the splitting of the whole non-toxic protein molecule to the higher non-coagulable but toxic forms (proteoses). Since the microchemical methods devised by Bang (1) and Folin (2) were described there are available no experiments dealing with this subject, and we have therefore undertaken the following experiments using the Folin method, which lends itself admirably to work of this kind.

The bactericidal and bacteriolytic property of fresh serum due to the action of complement and amboceptor has been assumed, more particularly by French and German writers, to be enzymotic in character and in the nature of a protease. There is, however, no experimental basis for this idea; indeed the fact that complement

* Received for publication, July 13, 1914.

action is not identical with a protease action can be demonstrated in a very simple experiment. When chloroform is added to fresh serum and thoroughly mixed, the complement is very rapidly destroyed; the serum protease, however, first becomes active under these conditions (Delezenne and Pozerski (3)). The activation under the influence of chloroform or other lipoid solvents is due to the fact that the antiferment is removed in this way. There should therefore be no confusion as to the non-identity of these serum constituents. If complement activity is enzymotic in character, we should rather seek to identify it with the lipases or allied enzymes. Thus the objection made that complement acts in direct proportion to the concentration, and is therefore unlike other ferments, fails in view of the fact that serum esterase acts in a like manner (Loevenhart (4)). And the ease with which lipoid solvents inactivate the complement finds an analogy in plant lipases which are either destroyed by lipoidal solvents or are in such intimate relation to the lipoids that they are taken up with them into the solvent. The substrate offered for complement action—red blood cells, animal cells, bacteria—is lipoidal in nature, the potentially lipoidal character of the limiting membranes of the corpuscles and animal cells being well established and rendered more than probable for bacterial cells. The fact that powerful proteolytic enzymes are without effect on such substrata is almost conclusive evidence that the surfaces of these substrata are not protein in nature. An observation of Metchnikoff (5) is significant in this connection. Metchnikoff, noting that the intestinal tract of certain insects (moths) was free from bacteria, an exception to the condition found in other insects, concluded that these insects must secrete a powerful fat-splitting enzyme to enable them to utilize waxes and fats readily, and surmised some connection between the character of this intestinal secretion and the freedom from bacteria.

EXPERIMENTAL.

The experiments, designed to determine the amount of proteolysis occurring during bacteriolysis, were carried out in the following manner. The organisms used, *Bacillus coli*, *Bacillus typhosus*, and staphylococci, were grown on agar in large bottles. They were

washed repeatedly with salt solution and used as fresh emulsions, or dried at low temperature and made up in a 1 per cent. emulsion as used. In either case the total nitrogen and the total non-coagulable nitrogen per cubic centimeter of the emulsion were determined for several samples. Immune sera were obtained by bleeding rabbits when the blood contained the greatest concentration of immune bodies. The immune sera so obtained were kept without preservatives on ice and were inactive. Fresh guinea pig serum was used as complement.

One cubic centimeter of the bacterial emulsion was measured into small centrifuge tubes, the various sera were then added, and digestion was permitted at 37° C. under toluol for varying periods of time. The tubes were then thoroughly centrifuged until the supernatant fluid was clear. The fluid was carefully pipetted from the bacteria, these were washed once with salt solution, and the fluid was added to that already withdrawn. The total nitrogen of the bacterial rest was then determined and also the total non-coagulable nitrogen of the supernatant fluid. The difference between this latter figure and the amount of total non-coagulable nitrogen in the serum controls gives the amount of nitrogen which has autolyzed, *i. e.*, the bacterial nitrogen digestion. The difference between the total nitrogen of the bacteria originally introduced and the amount recovered indicates the amount of bacteriolysis.

In using the relatively large amounts of organisms necessitated by the character of the work, the amount of serum is by comparison small, but the bacteriolytic effect is in most cases quite evident by the greater loss of bacterial nitrogen in the tubes containing the immune serum and complement.

The work has been carried out in duplicate and in some instances four or more determinations were made on corresponding mixtures. For the sake of brevity the protocols have been condensed and show only single determinations. The variations were seldom greater than a few hundredths of a milligram. The figures indicated in the protocols express nitrogen in milligrams.

In numerous experiments the bacterial rests remaining after serum treatment were digested by trypsin after determinations on similar mixtures showed the amount of substrate remaining. The

PROTOCOL I.

Action of immune serum and complement on bacteria and relation of resulting lysis to proteolysis.

Tube No.	Typhoid bacilli.	Colon bacilli.	Colon immune serum.	Complement.	Bacterial nitrogen recovered.	Loss in bacterial nitrogen.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coagulable nitrogen in supernatant fluid.
1	1.0 c.c.	Total nitrogen = 1.65 mg. per c.c.					Total non-coagulable nitrogen = 0.25 mg. per c.c.	
2	.....	1.0 c.c.	Total nitrogen = 2.80 mg. per c.c.				Total non-coagulable nitrogen = 0.38 mg. per c.c.	
3	1.0 c.c.	.....	.....	.....	1.4 mg.	0.25 mg.	0.2 mg.	.....
4	.....	1.0 c.c.	.....	.....	2.40 mg.	0.34 mg.	0.3 mg.	.....
5	1.0 c.c.	.....	.....	.....	1.51 mg.	0.14 mg.	0.2 mg.	Total nitrogen in supernatant fluid
6	.....	1.0 c.c.	.....	.....	2.34 mg.	0.46 mg.	0.53 mg.	
7	1.0 c.c.	.....	1.0 c.c.	2.0 c.c.	1.25 mg.	0.40 mg.	0.87 (−0.625) mg.	0.24 mg.
8	1.0 c.c.	.....	0.1 c.c.	2.0 c.c.	1.25 mg.	0.40 mg.	0.66 (−0.437) mg.	0.22 mg.
9	.....	1.0 c.c.	1.0 c.c.	2.0 c.c.	2.10 mg.	0.7 mg.	1.0 (−0.625) mg.	0.375 mg.
10	.....	1.0 c.c.	0.1 c.c.	2.0 c.c.	2.10 mg.	0.7 mg.	0.8 (−0.437) mg.	0.363 mg.
11	1.0 c.c.	.....	.....	2.0 c.c.	1.1 mg.	0.55 mg.	0.61 (−0.417) mg.	0.2 mg.
12	.....	1.0 c.c.	.....	2.0 c.c.	2.34 mg.	0.46 mg.	0.8 (−0.417) mg.	0.383 mg.
13	1.0 c.c.	.....	1.0 c.c.	.....	1.2 mg.	0.45 mg.	0.5 (−0.207) mg.	0.293 mg.
14	.....	1.0 c.c.	1.0 c.c.	.....	2.4 mg.	0.4 mg.	0.56 (−0.207) mg.	0.353 mg.
15	.....	.....	1.0 c.c.	.....	.....	.....	0.207 mg.	.....
16	.....	.....	.....	2.0 c.c.	.....	.....	0.417 mg.	.....

Action of trypsin on bacteria following immune serum and complement action.

Typhoid bacilli.	Colon bacilli.	Trypsin.	Bacterial nitrogen (substrate).	Nitrogen digestion.	Digestion.	
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....
1.0 c.c.	.....	0.1 c.c.	1.45 mg.	0.78 mg.	49%	Autolysis in salt solution
.....	1.0 c.c.	0.1 c.c.	2.4 mg.	0.75 mg.	31%	
1.0 c.c.	.....	0.1 c.c.	1.25 mg.	0.48 mg.	38%	Typhoid bacilli, colon immune serum complement
1.0 c.c.	.....	0.1 c.c.	1.25 mg.	0.50 mg.	40%	
.....	1.0 c.c.	0.1 c.c.	2.10 mg.	0.77 mg.	34%	Colon bacilli, colon immune serum complement
.....	1.0 c.c.	0.1 c.c.	2.10 mg.	0.508 mg.	24%	
1.0 c.c.	.....	0.1 c.c.	1.1 mg.	0.45 mg.	40%	Complement
.....	1.0 c.c.	0.1 c.c.	2.34 mg.	0.57 mg.	24%	
1.0 c.c.	.....	0.1 c.c.	1.2 mg.	0.55 mg.	45%	Immune serum
.....	1.0 c.c.	0.1 c.c.	2.4 mg.	1.0 mg.	40%	
.....	.....	.....	.....	.....	.....	Non-coagulable nitrogen in serum controls
.....	.....	.....	.....	.....	.....	

per cent. of digestion was determined by carefully acidifying and boiling and filtering through kaolinized hard paper filters to remove the coagulated protein. The solution of trypsin used was prepared each time from a dried preparation and used in such dilution that 0.1 of a cubic centimeter of the solution would digest two cubic centimeters of a 1 per cent. casein solution in two hours. Protocol I will serve as an example of such an experiment.

PROTOCOL I—A.

Autolysis in Physiological Salt Solution. Relation of Solution of Bacteria to Autolysis.

Tube No.	Typhoid bacilli.	Colon bacilli.	Total nitrogen in supernatant fluid after		Total non-coagulable nitrogen in supernatant fluid after		
			24 hrs.	48 hrs.	24 hrs.	48 hrs.	
1	1.0 c.c.		0.37 mg.				Solution.
2	1.0 c.c.			0.6 mg.			
3	1.0 c.c.				0.2 mg.		Autolysis.
4	1.0 c.c.					0.2 mg.	
5		1.0 c.c.	0.57 mg.				Solution.
6		1.0 c.c.		0.8 mg.			
7		1.0 c.c.			0.35 mg.		Autolysis.
8		1.0 c.c.				0.8 mg.	

Fresh typhoid and colon bacilli were used. The typhoid emulsion contained 1.65 milligrams of total nitrogen and 0.25 of a milligram of total non-coagulable nitrogen per cubic centimeter. The colon emulsion contained 2.80 milligrams of total nitrogen and 0.38 of a milligram of total non-coagulable nitrogen per cubic centimeter. Bacteria were permitted to autolyze in salt solution over night (tubes 3 to 6); with immune colon serum and fresh complement (tubes 7 to 10); with complement alone (tubes 11 and 12); and with immune colon serum alone (tubes 13 and 14). The total non-coagulable nitrogen in the sera used is shown in tubes 14 and 15, and these amounts, viz., $0.417 + 0.207 = 0.624$ of a milligram, $0.417 + 0.02 = 0.437$ of a milligram are, of course, to be deducted from the digestion mixtures. A parallel series of tubes was prepared, serum action permitted for an equal length of time, the bacteria washed and subjected to tryptic digestion for six hours.

It is apparent in observing the results obtained that the greatest

loss of bacterial nitrogen has occurred in the mixtures with colon bacilli, colon immune serum, and complement, indicating a marked lysis of these bacteria. There is, however, no corresponding increase in non-coagulable nitrogen obtained in the supernatant fluid, the amount so recovered in the 1.0 and 0.1 cubic centimeter dilutions of immune serum being respectively 0.375 of a milligram and 0.363 of a milligram, corresponding very well with the amount of non-coagulable nitrogen introduced originally with the bacteria, 0.38 of a milligram, and representing only a trace more than the amount recovered in the salt solution control (tube 4). There is obviously no relation here between the amount of lysis and the actual proteolysis. It is interesting to note that the greatest lysis of typhoid bacilli occurred in the mixture containing complement alone, although here, too, there is no corresponding increase in non-coagulable nitrogen. When the bacterial residues are digested by trypsin the percentage of digestion is found to be considerably less in all the tubes treated with serum than in the untreated controls (tubes 5 and 6), with the exception of the bacteria treated with immune serum (tubes 9 and 14). We have previously shown (6) that bacteria treated with normal serum become more resistant to tryptic digestion, because of their adsorption of serum antitrypsin.

In protocol I-A is shown the rate of solution and of autolysis of

PROTOCOL II.

Action of Typhoid Immune Serum and Complement on Typhoid Bacilli. No Demonstrable Proteolysis.

Tube No.	Typhoid bacilli.	Immune serum.	Complement.	Bacterial nitrogen recovered.	Loss.	Total nitrogen in supernatant fluid.	Gain in total nitrogen.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coagulable nitrogen.
1	1.0 c.c.	Total nitrogen = 1.1 mg., total non-coagulable nitrogen = 0.16 mg. per c.c.							
2	1.0 c.c.	Autolysis in sodium carbonate solution		0.66 mg.	0.44 mg.	0.43 mg.	.....	0.17 mg.	.....
3	1.0 c.c.	0.1 c.c.	1.0 c.c.	0.83 mg.	0.27 mg.	7 (−6.7) mg.	0.3 mg.	.....	.....
4	1.0 c.c.	0.1 c.c.	1.0 c.c.	0.83 mg.	0.27 mg.	.....	.....	0.55 (−0.39) mg.	0.16 mg.
5	.....	0.1 c.c.	1.0 c.c.	.....	.....	6.7 mg.	.....	.....	.....
6	.....	0.1 c.c.	1.0 c.c.	.....	.....	.....	.....	0.39 mg.	.....

these organisms when kept in salt solution at 37° C. under toluol. While solution has occurred in both cases, the autolysis is not equally rapid; indeed it did not occur at all in the typhoid emulsion in this case.

In protocol II a typhoid system was used, with fresh typhoid bacilli and typhoid immune serum. The total nitrogen per cubic centimeter was 1.1 milligrams; the total non-coagulable nitrogen was 0.16 of a milligram per cubic centimeter. Mixtures were made as usual, the reaction in this case being made distinctly alkaline to phenolphthalein, the control being placed in a sodium carbonate solution of equal alkalinity. The control lost 0.44 of a milligram of bacterial nitrogen, 0.43 of a milligram being recovered from the supernatant fluid, of which 0.17 of a milligram was non-coagulable, indicating a lysis without proteolysis in the control. It will be recalled that autolytic enzymes in general act best in a slightly acid medium. In the immune serum (tubes 3 and 4) there has been less lysis than in the alkaline control (loss of 0.27 of a milligram), and while this amount was recovered in the increase in total nitrogen in the supernatant fluid (0.3 of a milligram), the non-coagulable nitrogen had not increased (0.16 of a milligram). It is quite apparent that the solution of the bacteria, due in one case to the alkalinity of the fluid (tube 2), in the other to the lytic effect of the serum complement and immune body (tubes 3 and 4), is not accompanied by proteolysis.

If we permit the action of the immune serum and complement on the bacteria for a shorter period of time (4 hours at 37° C.),

PROTOCOL III.

Action of Typhoid Immune Serum and Complement on Typhoid Bacteria. Absence of Proteolysis after 4 Hours' Incubation.

Tube No.	Typhoid bacilli.	Typhoid immune serum.	Complement.		Bacterial nitrogen recovered.	Loss.	Gain in non-coagulable nitrogen
1	1.0 c.c.	Total nitrogen = 1.42 mg., total non-coagulable nitrogen = 0.2 mg. per c.c.					
2	1.0 c.c.	0.0 c.c.	0.0 c.c.	Autolysis	1.2 mg.	0.22 mg.	0.15 mg.
3	1.0 c.c.	0.0 c.c.	1.0 c.c.	.....	1.2 mg.	0.22 mg.	0.135 mg.
4	1.0 c.c.	1.0 c.c.	1.0 c.c.	.....	1.28 mg.	0.14 mg.	0.07 mg.
5	1.0 c.c.	0.1 c.c.	1.0 c.c.	.....	1.25 mg.	0.17 mg.	0.13 mg.
6	1.0 c.c.	0.01 c.c.	1.0 c.c.	.....	1.28 mg.	0.14 mg.	0.125 mg.

which is ample for the bactericidal effect of the serum even if not for actual bacteriolysis, there is practically no loss in bacterial nitrogen and no increase in non-coagulable nitrogen. As a matter of fact, there is much less splitting in the tube containing the greatest amount of antibody (tube 4, protocol III).

The fact that complement alone has no appreciable proteolytic effect even when bactericidal is shown clearly in protocol IV, in

PROTOCOL IV.

Effect of Complement on Colon Bacilli. No Resulting Proteolysis.

Tube No.	Colon bacilli.	Complement.	Reaction.	Bacterial nitrogen recovered.	Loss	Total nitrogen recovered in supernatant fluid.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coagulable nitrogen.
1	1.0 c.c.	Total nitrogen = 0.9 mg. per c.c.						
2	1.0 c.c.		Acid	0.39 mg.	0.51 mg.	0.5 mg.		0.17 mg.
3	1.0 c.c.		Alkaline	0.39 mg.	0.51 mg.	0.5 mg.		0.15 mg.
4	1.0 c.c.	1.0 c.c.	Acid	0.56 mg.	0.34 mg.		0.45(−0.23) mg.	0.22 mg.
5	1.0 c.c.	1.0 c.c.	Alkaline	0.44 mg.	0.44 mg.		0.3 (−0.23) mg.	0.07 mg.
6		1.0 c.c.					0.23 mg.	

which dried colon bacilli were used. Dried organisms are, as a rule, more easily digested by trypsin than freshly killed organisms, possibly due to alterations in the physical state of the external limiting membrane of the organisms. The bacillary emulsion contained 0.9 of a milligram of total nitrogen per cubic centimeter. When permitted to autolyze in salt solution, both acid and alkaline in reaction, the solution of the organisms was quite marked, only 0.39 of a milligram remaining after twenty-four hours. The total dissolved nitrogen recovered from the supernatant fluid was 0.5 of a milligram, of which 0.17 of a milligram was non-coagulable. When treated with fresh guinea pig complement the lysis was not so marked, being greatest in the tubes with an alkaline reaction; the proteolysis, on the other hand, was greatest in an acid reaction. The increase of 0.05 of a milligram over the amount in the control has, however, no significance, for such conditions never occur in the living animal.

In studying the effect of varying amounts of the antibody on the bacteria and their consequent resistance to tryptic digestion, we have at various times observed that bacteria treated with the largest amount of antibody became more resistant to the action of trypsin,

thus in a measure simulating the Neisser-Wechsberg phenomenon. Such an experiment is shown in protocol V.

PROTOCOL V.

Effect of Tryptic Digestion on Organisms Treated with Varying Amounts of Specific and Non-Specific Immune Serum and Complement.

Tube No.	Typhoid bacilli.	Staphylococci.	Typhoid immune serum.	Complement.	Trypsin.	Tryptic digestion.	Increase.
1	1.0 c.c.	.....	Total nitrogen = 1.4 mg., total non-coagulable nitrogen = 0.07 mg. per c.c.				
2	.....	1.0 c.c.	Total nitrogen = 0.715 mg., total non-coagulable nitrogen = 0.16 mg. per c.c.				
3	1.0 c.c.	.....	.....	.....	0.1 c.c.	0.625 mg.	.....
4	.....	1.0 c.c.	.....	.....	0.1 c.c.	0.15 mg.	.....
5	1.0 c.c.	.....	3.0 c.c.	0.3 c.c.	0.1 c.c.	0.54 mg.	−6%
6	1.0 c.c.	.....	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.625 mg.	.....
7	1.0 c.c.	.....	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.65 mg.	+2%
8	1.0 c.c.	.....	0.01 c.c.	0.1 c.c.	0.1 c.c.	0.55 mg.	−5%
9	.....	1.0 c.c.	3.0 c.c.	0.3 c.c.	0.1 c.c.	0.13 mg.	−3%
10	.....	1.0 c.c.	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.20 mg.	+7%
11	.....	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.24 mg.	+12%
12	.....	1.0 c.c.	0.01 c.c.	0.1 c.c.	0.1 c.c.	0.16 mg.	+1%

In this experiment an emulsion of fresh typhoid bacilli, total nitrogen 1.4 milligrams, and fresh staphylococci, total nitrogen 0.715 of a milligram, were used, permitted to remain with typhoid immune serum and complement for sixteen hours at 37° C., and then washed and digested with 0.1 of a cubic centimeter of trypsin solution. In the controls the typhoid digestion was 0.625 of a milligram, staphylococci digestion 0.15 of a milligram. It will be noted that with three cubic centimeters of the immune serum there was 6 per cent. less digestion than in the control; with one cubic centimeter an equal amount; with 0.1 of a cubic centimeter, a slight increase; while with 0.01 of a cubic centimeter, there was a drop to an amount less than the control. This action was not specific, for when staphylococci and the typhoid immune serum were used a similar and even more pronounced result was obtained. We are inclined to attribute this result to the adsorption by the bacteria of lipoids from the serum, which, if sufficient, as when larger amounts of serum are permitted to act, will protect the bacteria from

tryptic digestion, and overcome the alteration produced by the immune body which under ordinary circumstances seems to render the bacterial body less resistant to trypsin.

In order to avoid the presence of an excess of antibody we have sensitized bacteria with immune serum. They were then washed, treated with complement, washed again, and tryptic digestion of the sensitized and unsensitized bacteria was permitted.

PROTOCOL VI.

Effect of Sensitization of Typhoid Bacilli on Their Tryptic Digestibility.

Tube No.	Typhoid bacteria.	Sensitized typhoid bacteria.	Complement.	Trypsin.	Non-coagulable nitrogen in supernatant fluid.	Tryptic digestion.
1	1.0 c.c.		Total nitrogen = 0.55 mg., total non-coagulable nitrogen = 0.05 mg. per c.c.			
2		1.0 c.c.	Total nitrogen = 0.575 mg., total non-coagulable nitrogen = 0.00 mg. per c.c.			
3	1.0 c.c.			0.1 c.c.		0.162 mg.
4		1.0 c.c.		0.1 c.c.		0.172 mg.
5	1.0 c.c.		1.0 c.c.		0.31 mg.	
6	1.0 c.c.		0.1 c.c.		0.12 mg.	
7		1.0 c.c.	1.0 c.c.		0.35 mg.	
8		1.0 c.c.	0.1 c.c.		Lost	
9			1.0 c.c.		0.305 mg.	
10			0.1 c.c.		0.06 mg.	
11	1.0 c.c.		1.0 c.c.	0.1 c.c.		0.172 mg.
12	1.0 c.c.		0.1 c.c.	0.1 c.c.		0.15 mg.
13		1.0 c.c.	1.0 c.c.	0.1 c.c.		0.316 mg.
14		1.0 c.c.	0.1 c.c.	0.1 c.c.		0.25 mg.

In protocol VI there was no increase in autolysis of the two series of bacteria. This will be seen from tubes 5 to 8, the corresponding serum controls being tubes 9 and 10. The sensitized bacteria are much more easily digested by trypsin (tubes 13 and 14) than the unsensitized bacteria (tubes 11 and 12).

DISCUSSION.

In view of the established fact that the serum of practically all animals contains a markedly active antiferment against trypsin, leucoprotease, and the autolytic ferments,—a non-specific substance which in a neutral or slightly alkaline reaction inhibits protease

action of any kind and is essential to life by preventing the splitting of native proteins in the serum to toxic products,—we can hardly expect a result other than that noted in the above experiments; *i. e.*, the fact that bacteriolysis is not associated with a proteolysis. Jobling and Strouse (7) have called attention to the fact that the mere solution of bacteria should not be confused with proteolysis; the former might be due to purely physical factors, as when organisms are kept under lipoid solvents as preservatives. This distinction should be made with equal emphasis for the immunity reactions.

If bacteriolysis is not associated with proteolysis we must find some other explanation. This, we believe, should be sought in the relation of the lipoids of the bacteria and the lipolytic effect of the serum (Jobling and Bull (8)). That a physical change of some sort is induced in corpuscles treated with immune serum and complement has been shown by Eisner and Friedemann (9), and is indicated by the fact that bacterial organisms are rendered more digestible for trypsin. This might be due to (a) an alteration in the lipoidal surface membranes, rendering the organism more permeable for trypsin; (b) an oxidation, rendering the antiferments of the bacteria less active; or (c) the death of the organism (expressing, of course, a physical change) with resulting alteration of the balance of the bacterial protein and lipoids and consequent loss of antitryptic activity.

Our results might be considered to contradict directly the results obtained by several workers with the Abderhalden dialysis method, who have observed an increase in protein split products when bacteria were allowed to digest with immune serum (Voelkel (10)). These workers have ignored the presence of an excess of antiferment in the blood, assuming that proteolysis might take place under normal conditions with resulting splitting of bacteria. Inasmuch as Plaut (11) has recently shown that such absorbing substances as kaolin, starch, etc., give an equally positive reaction, and since de Waele (12) has just demonstrated the same fact by the simple means of precipitating the globulins of the serum, there can be no question that the idea of a specific protease action is unfounded. This view has also been taken by Kirschbaum and Köhler (13), who have been unable to obtain results by means of the dialysis method which

could in any way be interpreted as indicating a specific protease action upon bacteria. This, of course, does not hold true for the peptases, there being no antiferments in the serum against these ferments. Reasoning from a different line of experiments, based on the demonstration of the lipoidal nature of the antiferments (14), we have shown that serum from which the antiferment has been removed will autolyze (15) and that the antiferment can be removed by adsorption (16), after which there is, of course, no further impediment to either protease or peptase action. It is, therefore, more than probable that the dialyzable split products obtained by various workers from the bacterial digestion by immune serum represent digestion products brought about by an alteration in the balance of the ferment and antiferment when the latter is adsorbed by the bacteria. It is more easy to conceive that the soluble serum proteins are split when their antiferment is adsorbed than that the bacterial protein, protected by its lipoids and membranes, should be attacked.

CONCLUSIONS.

1. There is no demonstrable increase in non-coagulable nitrogen during bacteriolysis.

2. Bacteria treated with immune serum and complement are so altered that they are more readily digested by trypsin.

3. Bacteria treated with complement alone become more resistant to proteolysis.

4. Bacteria treated with an excess of immune serum and complement become more resistant to proteolysis.

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FURTHER EXPERIMENTS UPON THE EFFECTS OF EXTIRPATION OF THE THYMUS IN RATS, WITH SPECIAL REFERENCE TO THE ALLEGED PRODUCTION OF RACHITIC LESIONS.*

By ALWIN M. PAPPENHEIMER, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

Plates 22 to 25.

In a previous paper (1) dealing with the effects of extirpation of the thymus of white rats, the writer reached the conclusion that total removal of the gland at an early age was not necessarily followed by modifications of the normal development, nor by characteristic changes in the skeletal system.

While this article was in press, there appeared a short paper by Klose (2) embodying results at variance with these observations. Klose described briefly the results of thymus extirpations in pigs, goats, rats, and chickens. The rats were operated upon on the fourteenth day after birth. Some of the animals showed no results from the operation, and this failure is ascribed by Klose to the presence of accessory tissue within the thyroid gland. In rats without such accessory thymus tissue, death followed after eight to ten weeks, with symptoms of cachexia thymopriva. The bones in these rats showed severe rachitic changes. Most striking were the changes in the ribs, in which there was found a typical rosary, such as is seen in human rickets. Microscopically there was an extreme widening of the zone of growing cartilage, failure of calcification in the preparatory zone, great irregularity and distortion of the cartilage columns, and their penetration by blood vessels. Klose pictures further a dense spongiosa composed in the region of the costochondral junction almost wholly of osteoid, and an accompanying transformation of the marrow into "*Fasermark*." Similar but less striking changes were found also in the femora.

Magnini (3) has also published an article dealing with the effect of thymus extirpation upon white rats. Young rats, less than 60 or 65 gm. in weight, showed effects noticeable within a few days after the operation. There developed a progressive cachexia which terminated fatally after forty to fifty days. Older rats, up to 70 gm. in weight, survived for about two months, whereas still larger animals recovered and remained alive. By the injection of suitable doses

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of thymus extract, it was possible to prevent the ill effects of the thymus extirpation. The spleen was found enlarged in 75 per cent. of the thymectomized rats, but no microscopic changes were observed in liver, adrenals, hypophysis, or testicles. No mention is made of alterations in the skeletal system.

In view of such conflicting observations, it seems desirable to bring forward additional evidence in support of the conclusions reached in the writer's previous paper. It has been suggested[1] that negative results might be due to unrecognized lobules of accessory thymic tissue which had undergone delymphoidization or other involutional change making their identification difficult. While it is hard to refute this criticism absolutely, it may be said that in those cases in which accessory tissue has been present, there has been no difficulty in recognizing it as such and in differentiating it from lymphoid tissue. Further, the failure of such minute thymic rests to undergo hyperplasia in itself suggests that they are of no great functional significance.

As regards the alleged production of rachitic changes, it was pointed out that Klose, although aware of the occurrence of spontaneous rachitis in rats, had given no data by which one could exclude this disease as a possible explanation of his findings. At that time I had not met with rachitic lesions in rats. During the past spring, however, a number of rats have been studied which showed striking changes in the bones and teeth. Among these were rats which had been thymectomized at an early age, control rats of the same litters, and rats from the stock cages. It is believed that a detailed description of some of these animals will support the view that these alterations of the skeletal system are not due to the lack of thymus secretion, but are the result of the spontaneous disease first described by Morpurgo.

This paper, therefore, includes observations (1) upon litters in which complete thymectomy was not followed by alterations in growth, nutrition, or skeletal system; (2) upon litters in which both operated animals and controls showed lesions of the bones and teeth; and (3) upon unoperated animals from the stock cages, showing similar lesions. Through the coöperation of Professor W. J. Gies, calcium determinations of the bones and teeth were made in

[1] Personal communication from Professor J. Salkind.

a number of the rats, and the results of these determinations are published in a separate paper (4).

The operative technique has been fully described in the previous study. The completeness of the operation and the possible presence of accessory thymic tissue in the thymectomized rats was controlled in each case by an examination of serial sections of the entire neck organs, including the thyroid, and the upper half of the thoracic organs. The teeth and bones were decalcified in 5 per cent. nitric acid. The staining methods included Morpurgo's modification of Schmorl's thionin-picric acid method, which differentiates even in completely decalcified specimens between osteoid and completely calcified bone.

PROTOCOLS.

Litter U.—This litter consisted of seven rats, four of which were thymectomized on July 28, 1913. During the following weeks mother and young received protective inoculations of a *B. enteritidis* vaccine, since fatal infections with this organism were prevalent at that time.

Rat U2.—Emaciated, cyanotic, and anemic; died eight weeks after operation, having gained only 10 gm. It was eaten by the other rats and could not be examined.

Rat U7.—Control. Gained poorly, remained weak, and had a bloody discharge about the eyes and nose. It was killed sixty-eight days after operation. Weight at death 38 gm. No gross or microscopic lesions were found to explain the inanition.

Rat U4.—Thymectomy. This rat also failed to gain weight normally, but remained lively, and aside from poor nutrition, showed no evidences of illness. It was killed sixty-eight days after operation, rat U7 serving as control. Weight at death 34 gm.

Autopsy.—Negative, aside from emaciation.

Microscopic Examination.—Numerous necroses, old and recent, in the liver. There was absence of spermatogenesis. Serial sections of the neck organs showed three lobules of thymic tissue lying in front of the trachea. There was also a small accessory thymus lobule, below and external to the lower pole of the left thyroid lobe.

The remaining rats of the litter (U1, U3, U5, and U6) were kept under observation until January 28 and 29, 1914, when they were killed with ether.

Rat U1.—Thymectomy. On Dec. 6 a small piece of thymus from a rat of another litter (Z4) was implanted in the peritoneal cavity. No symptoms followed this operation, and no vestiges of the implanted fragment were found at autopsy.

Autopsy.—No gross visceral or skeletal changes.

Microscopic Examination.—A few small necrotic areas are seen in the liver,

with accumulations of wandering cells in the portal spaces. Splenic follicles are large, with a broad zone of paler mononuclear cells at the periphery. There are myeloid foci in the pulp, and numerous megakaryocytes. There is also one small collection of polynuclears. Near a lymph node in the region of the salivary gland, there is a small lobule of tissue, in part necrotic, in part composed of pale epithelioid cells. This can not be identified as thymus. Adherent to the pericardium there is a lobule of tissue measuring 500 by 150 by 160 microns, which is composed of oval or fusiform cells with pale nuclei, arranged about capillaries or irregularly distributed and infiltrated with lymphoid and plasma cells. It can not be identified as thymus tissue, since it shows no Hassall bodies nor larger epithelial cell complexes, and is probably granulation tissue.

Rat U3.—Thymectomy.

Autopsy.—No gross lesions in the viscera or skeletal system, save that there are several parasitic (?) cysts of the mesentery.

Microscopic Examination.—There are no infective lesions of the liver or spleen. The series of the neck organs is incomplete in that the upper and posterior portions are torn in places. The mediastinal structures are, however, intact. No thymus tissue is present, and no accessory glands are found.

Rat U5.—Control. There are suppurative foci in the middle lobe of the right lung. The spleen is enlarged. There are no other gross lesions.

Microscopic Examination.—No infective lesions in liver or spleen.

Rat U6.—Control.

Autopsy and Microscopic Examination.—Normal organs. There is active spermatogenesis.

The weights of the viscera and other data are summarized in the following table.

TABLE I.

Rat.	Duration of experiment.	Weight at operation.	Weight at death.	Length.	Weight of spleen.	Weight of testes.	Spermatogenesis.	Weight of adrenals.	Weight of thymus.	Remarks.
U1	185 dys.	14 gm.	97 gm.	151 mm.	0.400 gm.	♀	—	0.025 gm.	—	Complete thymectomy. Infective lesions.
U3	186 dys.	14 gm.	111 gm.	—	0.318 gm.	♀	—	0.030 gm.	—	Complete thymectomy.
U4	68 dys.	14 gm.	36 gm.	124 mm.	0.220 gm.	0.180 gm.	0	0.020 gm.	—	Incomplete thymectomy. Accessory thymus. Infective lesions.
U5	186 dys.	15 gm.	116 gm.	—	0.669 gm.	♀	—	0.036 gm.	0.178 gm.	Control. Suppurative foci in lungs.
U6	185 dys.	14 gm.	152 gm.	—	0.472 gm.	1.124 gm.	Active	0.030 gm.	0.202 gm.	Control. Normal organs.
U7	68 dys.	15 gm.	38 gm.	129 mm.	0.166 gm.	0.296 gm.	0	0.017 gm.	0.070 gm.	Control. Probably infected.

The bones and teeth were reserved for chemical examination and were not studied histologically. There were no gross changes.

The foregoing protocols show that rats may live without thymus tissue and in apparent health for at least 186 days. The differences in weight between the thymectomized rats and the controls are in all likelihood referable to infections, histological evidence of which was found in rats U1 and U4. Moreover, a sex difference of twenty-five grams at this age is to be regarded as normal. The great retardation of growth in rats U4 and U7 emphasizes the need of caution in attributing such effects to the removal of the thymus, rather than to incidental disease.

The following experiment offers further evidence in favor of the negative results of thymus extirpation.

Litter O.[2]—This litter consisted of six rats, upon four of which thymectomy was performed on July 17, 1913. During the following weeks all the animals received prophylactic injections of a vaccine of *B. enteritidis*. With the exception of one rat, O4, which developed bloody diarrhea and died after four weeks, all the rats of this litter gained steadily in weight and remained healthy. Rats O1, O2, and one of the controls, O5, were killed on Nov. 15, 1913, 120 days after the operation. Rat O3 and the remaining control, O6, were killed eleven days later. In none of the operated rats were there found rests of thymic tissue or accessory glands. It is unnecessary to give the detailed protocols, since a careful microscopic examination of the bones and teeth showed no abnormal conditions. The calcification of the dentine of the incisors is shown for comparison with that in rachitic rats in figures 1a and 1b.

The following protocols serve as examples of the second group, in which both operated and control animals showed distinct alterations in the bones and teeth.

Litter X.—This litter consisted of six rats, four of which were operated on Oct. 24, 1913, the remaining two being kept as controls. The estimated age at the time of operation was sixteen days.

Rat X1.—On Nov. 12, seventeen days after thymectomy, a portion amounting to about one fourth of the entire thymus of rat X6 was removed and implanted into the peritoneal cavity of X1. No ill effects followed the implantation until Dec. 1, when the rat was found lying upon its side, suffering from general convulsions. The forefeet were strongly flexed, the hind legs extended and paretic. The rat was killed with ether.

Autopsy.—Well nourished male; weight 36 gm. All operative wounds completely healed. Abdomen prominent, intestines distended. No peritonitis. Careful search shows no trace of implanted thymic tissue. Spleen small. No other visceral changes.

[2] Tabulated weights and measurements and weight chart of this litter are given in the previous article (1).

Cultures from heart blood remained sterile.

Microscopic Examination.—Liver normal, no necroses. Spleen follicles small. Many megakaryocytes. Kidney and pancreas normal. Testicular tubules lined with indifferent epithelium, showing no mitoses. No meningitis. Ganglion cells of motor area show no changes recognizable when stained with hematoxylin and eosin. Vesicles of thyroid large and filled with abundant colloid. Complete series of neck organs; every tenth section mounted. Later, intermediate sections including all suspicious areas were also examined. No undoubted masses of thymic tissue were found. A small nodule situated between the esophagus, descending aorta, and right bronchus, at first interpreted as a thymus rest, was subsequently concluded to be a lymph node. It showed a fairly distinct peripheral lymph sinus, and complete absence of large epithelial cell complexes and formed Hassall bodies. In and about the hypertrophic cervical lymph nodes were many eosinophilic polynuclear leucocytes. No mitoses were found in the parathyroids.

The bones and teeth were fixed in Orth's fluid and decalcified in 5 per cent. nitric acid.

No rachitic changes were present in the femur or tibia. The epiphyseal lines were narrow and regular; the layer of proliferating cartilage cells at the lower epiphysis of the femur were only two to three cells deep. The trabeculæ of the spongiosa were far apart and narrow, the osteoblasts flat and inconspicuous. The osteoid zone was extremely narrow, in many places not recognizable. The lymphoid marrow extends to the epiphyseal cartilage in the femur. The lower portion of the tibia is occupied by fatty marrow. In the lymphoid marrow of the femur, there are scattered areas of necrosis, similar to those found in infections with *B. enteritidis*.

Sections of the upper and lower incisors show nothing abnormal. The calcification of the dentine is complete, save for the normal narrow calcium-free zone.

The interesting point in this protocol is the onset of convulsions and a tetanoid condition after removal of the thymus and subsequent isotransplantation into the peritoneal cavity. It does not seem probable that the convulsions were due to intoxication following the absorption of the thymus tissue. At the time of death, the implanted fragment had apparently been entirely resorbed. Further, the experiment has been repeated in other rats, and in no case were similar symptoms observed. The presence of focal necroses in the bone marrow suggests an infection as a more probable cause for the nervous symptoms.

The absence of bone lesions, in spite of complete extirpation and lack of accessory tissue, is to be noted.

Rat X2.—On Nov. 12 a small fragment of thymus from rat X6 was implanted beneath the skin of the abdominal wall. The small nodule disappeared after a few days, and no remains of it were found at autopsy. There were no

convulsive symptoms or other evidence of illness following the implantation. The rat remained well, but was behind the remaining animals of the litter in size and weight. It was allowed to live 151 days after thymectomy, and was then killed together with control rat X5.

Autopsy.—Poorly nourished, eyegrounds pale, slight exophthalmos. Internal organs normal. In the thymic region there were five or six lymph nodes and fat: no gross remains of thymus.

The ribs show fairly marked deformity. The zone of growing cartilage is broad and translucent, and there is distinct nodular swelling at the junction of cartilage and bone. There are also several opaque, fusiform, whitish swellings along the shafts of the ribs. The femur is 5 mm. shorter than that of X5, the control, and thicker, but not noticeably deformed. The teeth show no gross changes, aside from a notching of the cutting surface of the upper incisors.

Microscopic Examination.—Neck organs cut in complete series. A small thymus IV is found at the lower pole of the right thyroid lobe. It contains no formed Hassall bodies, but there are many epithelial cells with large pale nuclei in the medullary portion. The smooth margin and the absence of follicles and peripheral lymph sinus easily distinguish the tissue from lymph gland. It can be traced through only six sections. There is a large accessory parathyroid on the left side, lying above and external to the thyroid. All parathyroids show numerous mitoses, one or two to each immersion field.

The liver is free from necroses. The splenic follicles are of moderate size and composed chiefly of large lymphocytes. The pulp shows groups of erythroblasts and fairly numerous eosinophilic mononuclears and megakaryocytes. There is evidently active hematopoiesis. In the lung there is hyperplasia of the peribronchial lymphatic tissue. Adrenal medulla shows intense chromaffin staining of cells and capillary contents. It does not differ from that of the control.

In the ribs the zone of proliferating cartilage cells is about 0.4 mm. in width (normal width about 0.1 mm.). By actual measurement with the projection apparatus, it is found to be about six times as broad as that of a normal control. It is composed of fourteen to sixteen swollen cells. The margin is uneven and irregular columns and groups of cartilage cells in which the ground substance stains like osteoid are found amongst the trabeculæ of the spongiosa. These are wide, irregular in their arrangement, and composed in the neighborhood of the epiphysis almost wholly of a homogeneous osteoid. Only the central portions take a bluish stain and show typical bone structure. The marrow spaces are narrow, and the marrow is fibrous. The osteoblasts are very high and conspicuous. The cortex is broad, and the marrow cavity of the shaft correspondingly reduced. The nodular swellings seen in the gross correspond to localized thickenings of the compact bone. The epiphyseal cartilage of the femur is very convoluted, and there are masses of swollen cells projecting amongst the trabeculæ of the spongiosa. The width of the proliferating zone varies. It is from six to twelve cells deep. The spongy bone is dense, the marrow spaces reduced in width.

The incisor teeth show pronounced changes in the calcification of the dentine, both in the upper and lower incisors. The essential feature is a defective and irregular calcification, with ingrowth of blood vessels into the calcium-free dentine. On the concave side of the upper incisor, there are alternating striæ of

calcified and calcium-free dentine. No lesions are found in the enamel epithelium. The changes in the teeth are illustrated in figures 2a, 2b, 2c, and 2d.

Rat X5.—Control, male. Killed with ether at the same time as X2.

Autopsy.—Well nourished, apparently healthy rat. No gross lesions of internal organs, ribs, femora, or teeth. Testes small, undescended.

Microscopic Examination.—Liver normal, no necroses. Splenic follicles about the same size as those of X2, and of similar structure. Mitoses are not very numerous. Megakaryocytes and eosinophils abundant in pulp. Adrenals show intense staining of chromaffin elements and of capillary contents. Testicular tubules for the most part devoid of differentiated spermatic cells, being lined with indifferent syncytium showing no mitotic figures. A few tubules show differentiation up to the formation of spermatids. The interstitial cells are hyperplastic. Thyroid vesicles slightly smaller, epithelium higher, than in the thyroid of X2. Parathyroids show mitoses (two or three in each section), but less numerous than in X2.

Two ribs were examined. One shows much more pronounced changes than the other. The lesions in the more severely affected rib are identical with those described in the rib of X2. The width of the growing cartilage is increased, and the disarrangement of the cartilage cells is extreme. There is an excessive amount of spongy bone in the region of the epiphysis, the trabeculæ having a wide osteoid margin. The marrow is fibrous in character. The femur also shows similar changes.

The incisors show a marked defect in the calcification of the dentine, with ingrowth of cells and blood vessels from the odontoblast layer, as shown in figures 3a, 3b, 3c, and 3d. The enamel epithelium of the upper incisors is interrupted at one point and folded in upon itself. The enamel in this region has fallen out.

The comparative weights and measurements of rats X2 and X5 are given in table II.

TABLE II.

Rat.	Duration of experiment.	Weight at operation.	Weight at death.	Length.	Spleen.		Adrenals.		Weight of testes.	Weight of thymus	Remarks.
					Weight.	Length.	Absolute weight.	Relative weight.			
X2	151 dys.	19 gm.	86 gm.	150 mm.	0.333 gm.	27 mm.	0.022 gm.	$\frac{1}{3640}$ gm	♀	—	Complete thymectomy. Small thymus IV.
X5	151 dys.	16 gm.	143 gm.	166 mm.	0.438 gm.	30 mm.	0.027 gm.	$\frac{1}{5300}$ gm.	0.374 gm.	0.143 gm.	Control. Cryptorchid.

There is a difference of weight of fifty-seven grams in favor of the control rat, and of sixteen millimeters in length. Both the operated rat and the control show marked lesions in the teeth and skeletal system.

The spontaneous occurrence of such lesions in the control rat

makes it highly improbable that they are in any way related to a loss of thymic function. Furthermore, if one is to ascribe the retardation in growth in rat X2 to the lack of thymus, one must assume that the presence of an accessory gland is not sufficient to ward off the effects. This would not coincide with Klose's assumption that such small lobules of accessory thymus tissue are capable of preventing the deprivation symptoms, and that the negative results of extirpation can be explained in this way.

The remaining rats of the litter, X3 and X4, and the control, X6, were killed 156 days after the operation.

Rat X3.—Large, well nourished male. Nothing abnormal noted in viscera, bones, or teeth. Many large lymph nodes in substernal region, but no gross remains of thymus.

Microscopic Examination.—Liver, spleen, adrenals, lungs, and kidneys normal. Testes show active spermatogenesis. Interstitial cells large, distinct, and numerous.

Neck organs cut in complete series. A lobule of thymus tissue is found lying along the trachea 1 cm. above the bifurcation. It is elongated and narrow, measuring approximately 1.5 by 0.3 by 0.075 mm. The thymus structure is distinct, although there are few fully formed Hassall bodies. At one pole are several vesicles lined with cuboidal epithelium, and containing basic staining colloid material. The right parathyroid lies external to the thyroid, and is irregular in shape, partially encircling the carotid. Mitoses are not infrequent.

In the upper incisors the calcification of the dentine is practically complete on the convex surface throughout the whole length. Near the base of the tooth, the border of the calcified portion is slightly wavy, and there are a few ingrowing blood vessels. The concave edge also shows good calcification of the dentine, although in the middle portion there are a few calcium-free streaks, and in places the calcium is deposited in globular form. The odontoblast layer is well formed through the entire extent of the pulp cavity on both sides. The enamel is wanting in the sections, but the emaloblasts are normal.

Normal chondrocostal junctions in several ribs examined. There are four to eight growing cartilage cells, regularly disposed. The osteoblasts are well formed. There is no excess of osteoid tissue.

Rat X4.—Thymectomy.

Autopsy.—Fairly well nourished female. No changes in internal viscera. No macroscopic remains of thymus. Definite lesions are seen in the ribs, similar to those described in X2. The chondrocostal junctions are swollen, and there is sharp angulation of the lower ribs. The zone of growing cartilage is broad, and the adjacent part of the shaft for a distance of about 2 mm. is greyish white and soft. Four of the ribs show on the pleural surface, about 1 cm. from the junction with the cartilage, pearly, cartilaginous swellings. Teeth and femora show no gross changes.

Microscopic Examination.—Liver, kidneys, and adrenals normal. Splenic follicles slightly smaller than those of the control, X6. Few mitoses. Many

eosinophils and much pigment in pulp. Lungs show many eosinophils in edematous tissue about larger blood vessels.

The upper incisors show changes similar to those described in rats X2 and X5, but somewhat less marked. The defective calcification of the dentine, on the convex surface, near the base of the tooth, is marked, about one third of the entire width being entirely calcium-free. In the middle portion, about two fifths of the dentine is uncalcified. Here, and also towards the tip, the calcium is deposited in laminæ. There is an abundant ingrowth of capillaries from the odontoblast layer. On the concave side, near the base, half the dentine is calcium-free. Towards the middle and distal portion, the calcification is still more incomplete, and in the form of alternating calcium-free and calcium-containing layers. The lower incisors show similar changes, especially marked on the concave surface.

The growing cartilage of the ribs presents an even convex surface towards the shaft. The rows of cartilage cells are six to ten cells deep. The spongiosa is composed of very broad trabeculæ with wide osteoid zones, and high osteoblasts. The pearly swellings observed in the gross correspond to localized thickenings of the compact bone.

The zone of growing cartilage of the femur at the lower epiphysis is convoluted and slightly increased in breadth, but not markedly irregular. The spongiosa is formed of coarse trabeculæ with a relatively broad osteoid zone and distinct osteoblasts.

Neck organs, cut in complete series, show no thymus tissue, with the exception of a minute accessory lobule below the inferior pole of the right parathyroid gland and lying within the capsule of the thyroid. This is composed of large cells with very pale nuclei, between which are scattered small cells of the lymphoid type. The lobule contains also a few eosinophils, and abundant brownish pigment. There are no Hassall bodies or large epithelial cell complexes. The tissue can be traced through only five sections. There are two parathyroids, both within the thyroid. The epithelial cells show fairly numerous mitoses, two or three to each section. The thyroid vesicles are small, save at the surface of the gland; the cells are columnar, but not infolded. The colloid is abundant, but vacuolated.

Rat X6.—Control.

Autopsy.—Large, well nourished rat, showing no changes in the internal organs. There is slight beading of the ribs, with a broad greyish zone adjacent to the cartilage. The femora and teeth appear normal.

Microscopic Examination.—Liver, kidneys, and adrenals normal. The splenic follicles are slightly larger than those of rats X3 and X4. Mitoses are not numerous. In the pulp are many erythroblasts and eosinophils. Some of the testicular tubules show active spermatogenesis with formed spermatozoa in the lumina. Most of the tubules, however, are lined with indifferent epithelium showing no mitoses. Interstitial cells are somewhat more abundant than in the testis of rat X4.

In the upper incisors the odontoblasts are well formed, extending through the entire length of the pulp. Calcification of the dentine is not abnormal, save that the odontoblast layer is rather wavy. The uncalcified predentine is narrow.

Ribs and femur show nothing abnormal.

The comparative weights and measurements of rats X3, X4, and X6 are summarized in table III.

TABLE III.

Rat.	Duration of experiment.	Weight at operation	Weight at death.	Length.	Spleen.		Adrenals.		Weight of testes.	Remarks.
					Weight.	Length.	Absolute weight.	Relative weight.		
X3	156 dys.	19 gm.	195 gm.	199 mm.	0.447 gm.	31 mm.	0.027 gm.	$\frac{1}{7240}$	1.382 gm.	Incomplete thymectomy.
X4	156 dys.	16 gm.	110 gm.	165 mm.	0.311 gm.	31 mm.	0.028 gm.	$\frac{1}{3947}$	♀	Complete thymectomy. Minute thymus IV.
X6	150 dys.	20 gm.	180 gm.	205 mm.	0.542 gm.	34 mm.	0.021 gm.	$\frac{1}{8571}$	0.440 gm.	Control. Hypoplasia of testicles.

Rat X4 offers a further illustration of the fact that rats may survive complete extirpation for many months without becoming cachectic. It seems improbable that the minute accessory gland, less than fifty microns in diameter, could take over the functions of the entire thymus without showing evidence of active hyperplasia.

The rachitic lesions in this rat were marked, and as will be evident from subsequent protocols, this disease in rats is frequently, though not always, accompanied by a retardation of growth. There is, therefore, little ground for attributing the diminution in size and weight, as compared with the control, to the lack of thymus. The normal sex difference must also be taken into account at this age.

Litter Y.—This litter, consisting originally of eleven rats, was born in the laboratory of stock animals, all the rats previously studied having been obtained from Granby, Mass. The rats were undersized, and stood the operation poorly, only three thymectomized and three controls surviving.

Rat Y2.—Thymectomized Oct. 27, 1913. Weight 10 gm. Gained slowly and remained undersized. Killed on Mar. 13, 1914, 137 days after extirpation of the thymus.

Autopsy.—Internal organs normal save for suppurative foci in the lower lobe of the right lung. Testes large and descended. No alterations of the bones and teeth were recorded.

Microscopic Examination.—Lungs show bronchiectases and bronchopneumonia. Active spermatogenesis. Interstitial cells not numerous. Liver, adrenals, and kidneys normal. Spleen shows active blood formation.

Extirpation of thymus probably complete. The only suspicious tissue lies in a lobule of fat near the hilus of the left lung, where there is a circumscribed collection of mononuclear cells with a few larger elements, which can be traced

through sixteen sections. There are no epithelial cell complexes or Hassall bodies, but the tissue does not have a peripheral lymph sinus or follicles. It is probably a newly forming lymph node. The thyroid is composed of large vesicles with abundant colloid and flat epithelium. In the fat tissue external to it, are a few closed ducts lined with high cylindrical epithelium (postbranchial ducts).

The line of growing cartilage of the ribs is broad, with a convex, irregular surface directed towards the shaft. It is composed of eighteen to twenty cells in rows, with masses of cartilage cells projecting amongst the trabeculæ of the spongiosa. These are numerous, broad, with a wide osteoid margin, and high conspicuous osteoblasts. The marrow spaces in the region of the cartilage are narrow, the vessels hyperemic and surrounded by spindle cells.

Upper epiphysis of tibia. No definite rachitic changes. The zone of growing cartilage is slightly narrower than that of the control. The cells are regular in their arrangement, as are the trabeculæ. Bone formation is very active. The osteoid zone about the trabeculæ is slightly increased in width. This is especially clear in the epiphyseal center of ossification.

The epiphyseal line of the femur is regular on the whole, but there are a few projections of cartilage amongst the trabeculæ of the spongiosa. The osteoid is increased in amount.

The calcification of the dentine of both upper and lower incisors is somewhat defective, but there is no vascularization of the calcium-free dentine. The enamel epithelium is not altered.

Much more striking were the skeletal and dental changes in rat Y8, the protocol of which follows.

Rat Y8.—Thymectomy on Nov. 1, 1913. Weight at operation 14.6 gm. Following the operation, and until the rat was killed on Mar. 2, 126 days after extirpation of the thymus, it lagged far behind the remaining rats of the litter in weight and size. The hair was rough and sparse; the head seemed relatively large as compared with the body. On Jan. 17, it was noted that the upper incisors were very long and thick as compared with the lower, which were small and delicate. On Feb. 28 the right upper incisor was broken off just beyond its emergence from the gum.

Autopsy.—Poorly nourished, stunted male. Weight 42 gm. The internal viscera were normal, with the exception of the right lung, which was atelectatic in its upper portion. No gross remains of thymus. The thorax is greatly deformed by the presence of a deep groove along the line of the chondrocostal junctions. From the pleural surface there are seen, especially on the lower ribs, nodular protuberances at corresponding points. There is thus a pronounced rachitic rosary. The bones of the lower extremities are softer and more pliable than normal, and there is distinct swelling about the epiphyses of the knee-joints.

Cultures from the heart blood and spleen on slant agar remained sterile.

Microscopical Examination.—Liver free from necroses. Splenic follicles large and sharply circumscribed. Many of the lymphoid cells are of the large mononuclear type. Mitoses are scarce, and there are no distinct germinal centers. In the pulp there are many islands of normoblasts and groups of myelocytic eosinophils. Adrenals show the usual intense chromaffin staining. Testicular tubules are lined with four or five rows of cells, most of them spermatoblasts

in active division. There are a few spermatids, but no ripe spermatozoa. The appearance is that of a testis just before the onset of spermatogenesis. Some of the bronchi contain dense collections of eosinophils. The pulmonary arteries are thickened, their lumina contracted, the muscular coat appears hypertrophied. Some of the vessels are practically obliterated.

Complete series of neck organs. There is no tissue which can be identified as thymus. There are no accessory thymus lobules. The parathyroids are large; no mitoses are found. Thyroid shows usual structure with abundant colloid.

The upper incisors were sectioned through the fractured stump. The pulp is hyperemic and largely necrotic. The calcification of the dentine is both irregular and incomplete. The line of junction between odontoblasts and predentine is sinuous, and in many places the odontoblast cells penetrate the uncalcified tissue. The calcified portion of the dentine is laminated, the calcium being in the form of coarse globules. In another section there are alternating zones of calcified and calcium-free dentine. Isolated cells and occasionally capillary loops reach the outer, imperfectly calcified zone. Sections through the unfractured upper incisor show also defective calcification. Near the root, there is the same stratification as in the fractured tooth. In the middle portion, just after it emerges from the socket, the section passes outside the pulp canal. In this region there is a large area of calcium-free dentine. Near the convex surface, within the calcified strata, there are a number of globular yellowish masses. In sections stained with thionin-picric acid, it is seen that the dentine filaments are interrupted at the site of these globular deposits. Near the root, the congested pulp vessels penetrate the uncalcified dentine as papillary ingrowths. About one half of the dentine on the convex surface of the lower incisors is free from calcium. The odontoblasts are poorly formed and irregular. There is no marked ingrowth of blood vessels. On the concave side the calcification is still more imperfect.

The zone of growing cartilage of the humerus at the lower epiphysis is widened, and irregularly prolonged amongst the trabeculæ. These are very broad and the marrow spaces correspondingly narrow. The trabeculæ are composed in the neighborhood of the epiphysis of a narrow, ossified central portion, distinctly laminated, with angular bone corpuscles, surrounded by a very broad, homogeneous, osteoid zone in which the nuclei of the bone corpuscles are larger and more rounded, and quite irregularly distributed. The osteoblasts are conspicuous and well formed. There is no increased osteoclastic resorption. The changes at the lower epiphysis of the femur are similar in character, but more pronounced than in the humerus. Numerous sections from four different ribs show similar changes. There is marked deformity at the junction of cartilage and bone, where the rib measures 3 mm. in diameter. The junction of the cartilage with the shaft is in most places sharply marked off. There is a large central mass of hypertrophic cartilage, in some places twenty-five cells deep, and two shorter lateral spurs. Near the pleural surface, smaller irregular groups of cartilage cells are surrounded by osteoid-staining matrix. The adjacent cancellous bone is composed of stout trabeculæ formed entirely of pink staining osteoid, separated by very narrow clefts and spaces. They are ranged, in the region where the rib is sharply angulated, at right angles to the cartilage columns.

The distribution of the bone corpuscles through this mass of osteoid is haphazard. Farther along the shaft, the trabeculæ of the spongy bone follow the long axis of the rib. They are all greatly thickened, as is the corticalis, and well calcified only in their central portions. In thionin-picric acid-phosphotungstic acid-stained preparations, the broad osteoid, blue staining zone appears with great distinctness. The marrow cavity is much reduced in width. Near the epiphysis the marrow is fibrous; further along, it has the normal lymphoid appearance.

Two control rats of the litter, Y7 and Y9, showed alterations in the teeth and bones comparable with those observed in the thymectomized rats, though somewhat less pronounced. A third control, Y10, which had complete visceral transposition, was not histologically examined.

Rat Y7.—Control. Killed Apr. 7, 1914. Poorly nourished female, weighing 115 gm. Internal organs show no lesions, with the exception of the lung. The right middle lobe is converted into a mass of cheesy abscesses, separated by scar tissue. The ribs show slight beading at the chondrocostal junctions, with angular deformity in the region of the diaphragmatic attachment. The zone of growing cartilage is broad. No gross changes in femora or teeth.

Microscopic Examination.—Internal viscera, aside from lung lesions, show nothing abnormal.

In the upper incisors calcification of the dentine is imperfect, both on the convex and on the concave surfaces. The odontoblast layer is broad, but ill defined, and on the concave side cells are seen to penetrate the calcium-free dentine for varying distances. Both surfaces of the lower incisors show alternating layers of calcium-containing and calcium-free dentine. The calcium is deposited in the form of coarse globules.

TABLE IV.

Rat.	Duration of experiment.	Weight at operation.	Weight at death.	Length.	Spleen.		Adrenals.		Weight of testes.	Remarks.
					Length.	Weight.	Absolute weight.	Relative weight.		
Y2	150 dys.	10 gm.	85 gm.	145 mm.	26 mm.	0.220 gm.	0.013 gm.	$\frac{1}{6538}$	1.483 gm.	Complete thymectomy.
Y8	121 dys.	14.5 gm.	42 gm.	127 mm.	25 mm.	0.312 gm.	0.020 gm.	$\frac{1}{2100}$	0.332 gm.	Complete thymectomy.
Y9	150 dys.	16 gm.	118 gm.	170 mm.	32 mm.	0.380 gm.	0.030 gm.	$\frac{1}{1033}$	1.980 gm.	Control. Abscesses of lungs.
Y7	168 dys.	16 gm.	115 gm.	157 mm.	34 mm.	0.333 gm.	0.043 gm.	$\frac{1}{2674}$	♀	Control.

The growing cartilage of the femur is narrow, being composed of only two to four rows of cells in the lower epiphysis. Both in the compact and in the spongy bone, the osteoid margin is very broad and distinct, especially about the

perforating vessels. The ribs show no deformity in the sections examined, but there is a distinct increase in the width of the osteoid zone about the trabeculæ.

Rat Y9.—Control, male. Killed Mar. 13, 1914. Weight 118 gm. There were no lesions of the internal viscera. The alterations of the teeth were insignificant (slight lamination of the calcium on the convex surface of the lower incisors), but there was distinct irregularity and thickening of the cartilage at the lower epiphysis of the femur and at the upper epiphysis of the tibia. The single rib examined was practically normal.

The comparative weights and measurements of litter Y are given in table IV.

To the foregoing protocols of thymectomized and control rats may be added the brief description of two unoperated rats taken from the stock cages, which showed lesions in the bones and teeth identical with those described above.

The first of these (rat 50) was greatly stunted; there was a marked disproportion between the size of the head and that of the body. The approximate weight was 50 gm. The hair was rough and sparse. The teeth were somewhat chalky, but not greatly altered. There was an extreme deformity of the thorax (figure 5), resembling that noted in rat Y2. There was also marked kyphosis and lateral curvature. There were many whitish nodules along the shafts of the ribs, especially in the paravertebral lines. The ribs were very soft, and on cross-section the marrow cavity seemed almost obliterated owing to the great thickening of the cortex of the bone. At the chondrocostal junctions were nodular projections several millimeters in thickness. The femora were short, and very broad in the region of the epiphyses. The upper third of the tibiæ was bowed anteriorly.

Microscopic Examination.—The superficial layer of dentine of the upper incisors is well calcified; in the middle zone, the calcium is in the form of globular masses of large size, and the inner zone, comprising about one third of the total thickness of the dentine, is calcium-free. Towards the apex the calcification is more complete. The lower incisors exhibit more striking changes, especially on the concave surface where about half the dentine is free from calcium. The vessels of the pulp are extremely congested, and the odontoblast layer is broken up in many places by fresh capillary hemorrhages. Towards the apex of the tooth, the layer of odontoblasts becomes entirely disorganized, with the formation of numerous ragged cavities, possibly the effect of gas formed during decalcification. The enamel epithelium is intact save in one place, where the cells are destroyed by hemorrhage.

The zone of proliferating cartilage of the ribs is composed of irregular masses of cells which fail to show the normal alignment into columns, and which extend a variable distance into the adjacent trabeculæ. The neighboring part of the rib is composed of a knob of anastomosing columns separated by very narrow Haversian spaces. The distinction between osteoid and calcified bone is not sharply brought out in the sections, but the rounded form of the bone corpuscles and their highly irregular distribution suggest deficient calcification. The osteoblasts form a continuous flat row covering the trabeculæ. There is no increased

lacunar resorption. Beyond this knob of tissue the bone is sharply bent upon itself. A tongue of cartilage grows into this angle from the external surface of the periosteum. The cortex of the rib is greatly thickened and formed of parallel trabeculæ which encroach upon the medullary cavity, which is correspondingly narrowed. The marrow is normal, save in the region of the junction where it is fibrous (figure 6).

In order to determine the possible infectious character of this disease, four half grown and apparently healthy rats were inoculated with a suspension of bone marrow from rat Y8. One of the inoculated rats, killed twenty-eight days after subcutaneous injection, showed striking alterations in the bones and teeth. There was in this rat no gross deformity of the thorax, but an evident widening of the translucent zone of proliferating cartilage, and the adjacent bony part of the ribs was opaque and slightly thickened. The femora were broad and thick, and so soft that they could easily be cut with scissors. The tibiæ were bowed anteriorly.

Changes in the teeth were first noted four days before the animal was killed. The basal portions of the upper incisors were found to be distinctly swollen, translucent, greyish, and rounded, sharply marked off from the distal third which was opaque and chalky white. During the next few days, the swelling and translucency extended almost to the tip of the tooth. The lower incisors were also less opaque than normally.

Microscopic Examination.—No changes were found in any of the internal viscera. The marked defect and irregularity of the dentine is shown in figures 4a, 4b, 4c, and 4d. It is seen that on the convex surface of the upper incisors the irregularity in the arrangement of the odontoblasts is such that these have apparently given rise to predentine both on their external and internal surfaces. They are thus disposed in a row on both sides of which is uncalcified dentine, and are connected by numerous cell strands with the pulp. The concave side of the tooth is calcium-free throughout its entire extent, and is pierced in many places by isolated spindle cells and occasionally by capillaries. The changes in the ribs and femora consisted merely of an increase in the width of the osteoid margins. No definite alterations were found in the cartilages.

DISCUSSION.

The foregoing protocols show that lesions of the bones and teeth were observed in thymectomized rats, in controls of the same litters, in stock animals, and in a rat inoculated with bone marrow from a thymectomized rat with lesions of the skeletal system.

There is little question that these lesions resemble closely those described by Morpurgo (5), in 1900, as infective osteomalacia of white rats. In his subsequent investigations, extending over many years, Morpurgo showed that in young animals, the spontaneous disease took the form of rachitis.

From the spinal cord of a spontaneously diseased osteomalacic rat, and later, from the spleen, liver, kidneys, and bones, Morpurgo isolated a Gram-positive diplococcus with a tendency to form chains in fluid cultures. With pure cultures of this microörganism, Morpurgo was able to produce rachitic lesions in young rats, and osteomalacia in adult rats in which epiphyseal growth had been completed.

The clinical course of the disease varied in different rats, in part owing to variations in the virulence of the cultures, in part apparently to variations in the susceptibility of individual animals. In some cases the rats became greatly emaciated, and died from the disease. Other animals lived for over a year, in which case the bone lesions showed evidence of healing.

The deformities produced also varied widely in different rats. They were found especially in the vertebral column, the thorax, the scapulæ, the posterior extremities, and the pelvis,—more rarely in the anterior extremities and the maxillary bones. The vertebral column was lordotic and scoliotic in the dorsal region, kyphotic in the sacral and cervical portions. The thorax was much depressed along the insertion of the pectoral muscles, and along the line of depression the ribs were enlarged in consequence of callus formation following infractions or complete fractures. Small beads of callus were also seen along the paravertebral line, or scattered irregularly along the course of the ribs. The cartilages were also tortuous, nodose, and yellowish.

The femora were rarely curved or bent, and only when the presence of callus indicated a previous fracture. The diaphysis was often greatly enlarged, the medullary cavity eccentric or narrowed. The tibia was often bent at an angle between the upper and middle thirds.

The microscopic examination of the bones disclosed a picture which was identical with that seen in human rickets or osteomalacia. Schmorl and others have accepted the morphological identity of the lesions in the rat with those of human rachitis.

I have not found in Morpurgo's numerous papers any reference to changes in the teeth. Weichselbaum (6), however, reported finding lesions in the teeth in three spontaneously rachitic rats, which he describes as follows:

"The dentine of the incisors nowhere shows a uniformly calcified zone, but the zone adjacent to the odontoblasts, *i. e.*, the younger layer, was completely bereft of calcium, and thus appeared pale red in hematoxylin-eosin preparations, whereas the older layers were either wholly calcium-free or unevenly calcified, in which case they contained numerous blue dentine globules separated by pale, so called interglobular spaces. One sees further that from the odontoblast layer, capillary loops penetrate more or less deeply into the dentine. In one of the two rats one of the upper teeth was broken off."

Weichselbaum calls attention to the close resemblance of these changes found in the spontaneous rachitis of rats, to the lesions found by Fleischmann (7) in the teeth of human rachitic subjects, and especially to the dental changes described by Erdheim (8) and Toyofuku (9) as following parathyroidectomy in rats.[3] Hohlbaum (10), also, confirming Erdheim's findings, noted in two spontaneously affected rachitic rats identical lesions in the teeth. Furthermore, Iselin (11), in his study of the effects of parathyroid extirpation, found rachitic-like changes in the epiphyses of those rats which survived for a longer period, and attributed them to the loss of parathyroid function.

Defective calcification of the dentine, and penetration of the uncalcified tissue by blood vessels and isolated cells from the odontoblast layer, regarded by Erdheim and his successors as characteristic of parathyroid deprivation, were observed also in many of the rats described above. In one rat, there occurred a spontaneous fracture of the tooth. Less marked in my rats was the infolding and disarrangement of the emaloblast layer; but this appears to be of secondary importance also in the lesions attributed to parathyroidectomy, and when present is probably due to mechanical causes.

The question arises, therefore, whether Erdheim, who was familiar with the spontaneous rachitic lesions of rats, was justified in attributing these changes to parathyroid insufficiency. Erdheim (12) has brought forward his transplantation experiments as crucial proof of the part of the parathyroids in normal calcification. The "*Transplantations-strcife*" is believed to correspond exactly in its localization with the period of functional activity of the implanted parathyroid tissue.

This would be entirely convincing were it not for the fact that such alternating layers of calcium-containing and calcium-free den-

[3] This resemblance was accepted by Erdheim, who studied Weichselbaum's specimens.

tine not infrequently occur as spontaneous rachitic lesions. They are associated, no doubt, with remissions and exacerbations of the disease, such as have been described by Morpurgo. Figure 3 illustrates the striate deposition of the calcium in the incisor of a spontaneously rachitic rat.[4]

Without wishing to discredit the work of Erdheim, supported as it is by other facts indicating an influence of the parathyroids upon calcium metabolism, one cannot but emphasize the identity of these lesions attributed to the loss of parathyroid function, with those occurring in the spontaneous rickets of rats.

As regards the infectivity of the disease and the nature of the inciting organism, studies made in coöperation with Dr. J. G. Hopkins have not as yet led to conclusive results. The occurrence of rachitic lesions in a rat, examined twenty-eight days after inoculation of bone marrow from a spontaneously rachitic animal, is of doubtful significance, since many of the stock animals killed at this time were found to be rachitic. The recent experiments of Koch (13), in which typical rachitic lesions were produced in puppies by the intravenous injection of streptococci, indicate that the infective factor has to be reckoned with in experiments dealing with the effect of extirpation of ductless glands upon bone development in dogs as well as rats.

Sufficient evidence, we believe, has been brought forward to show that the bone changes described by Klose are in no way related to the loss of the thymus gland, but occur as the result of spontaneous disease. It is difficult indeed to understand why Klose should believe that his "experiments on rats acquire fundamental significance in view of the experiments of Morpurgo";[5] and why, in the same article, he should imply that rats are amongst those species which are practically immune to spontaneous rickets.[6]

As regards alterations in the weights and microscopical structure of the adrenals, testes, and spleen, which might point to a correlation between the thymus and these organs, nothing has been found to

[4] This appearance is pictured in figure 4, plate 6/7, of Toyofuku's paper (9), where it occurred in a rat, forty-six days after parathyroidectomy, in which no transplantation had been performed.

[5] Klose, *loc. cit.*, p. 4.

[6] Klose, *loc. cit.*, p. 7.

modify the negative conclusions reached in the previous paper. An analysis of the additional figures given therefore seems unnecessary.

CONCLUSIONS.

Rachitic changes in the bones and teeth occur in young albino rats as the result of spontaneous disease, possibly of infective origin. This disease is often, but not always, accompanied by an arrest of growth and malnutrition.

There is no valid reason for attributing such lesions to the loss of thymic function. In some of the rats showing the disease, an unusual number of mitoses were found in the parathyroid glands, but no evidence of injury to these structures.

It has not been found possible to confirm the statements of Klose and Magnini as to the fatal effects of complete thymus extirpation in rats.

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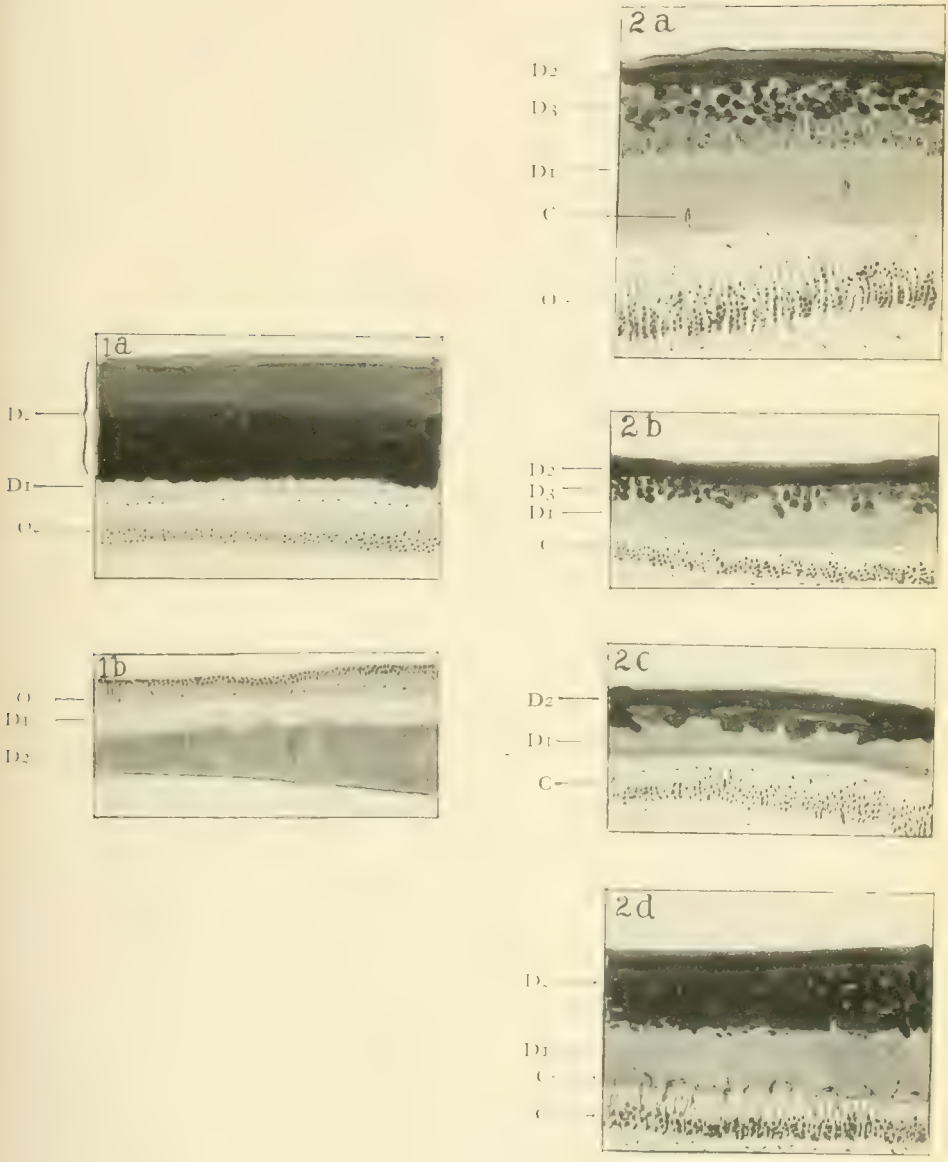
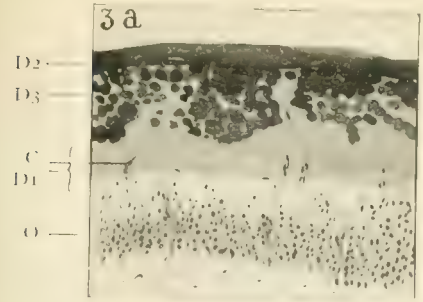
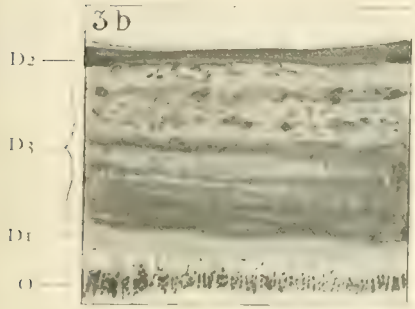
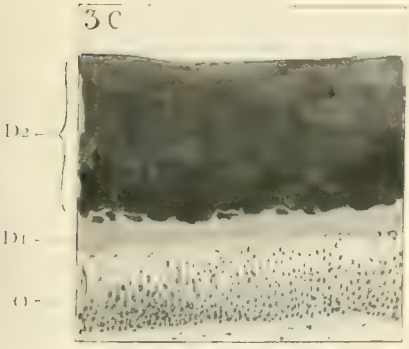
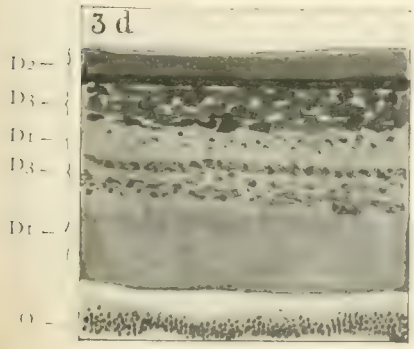


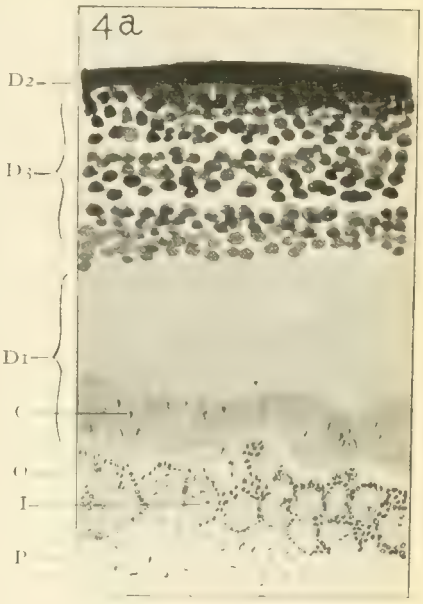
(Pappenheimer: Effects of Extirpation of Thymus in Rats.)

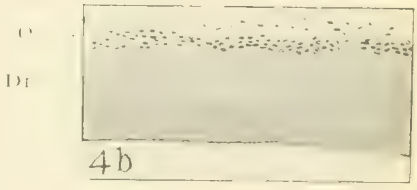


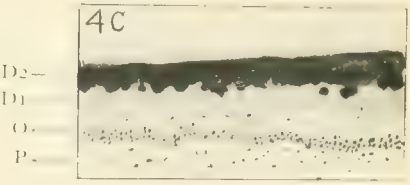


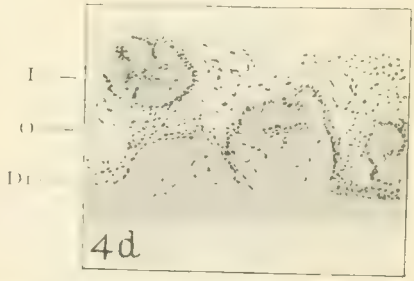












Puppenheim: Effects of Extirpation of Thymus in Rats.

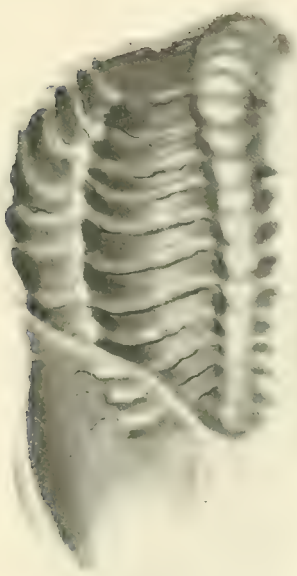


FIG. 5.

(Pappenheimer: Effects of Extirpation of Thymus in Rats.)

Fig. 6

(Pappenheimer: Effects of Interruption of Thymus in Rats.)

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EXPLANATION OF PLATES.

Plate 22.

Fig. 1a. Rat O1. Thymectomy. Killed after 120 days. Upper incisor, convex side. D1 = calcium-free dentine. D2 = calcified dentine. O = odontoblasts.

Fig. 1b. Same tooth, concave side. Normal relations between calcified and uncalcified dentine.

Fig. 2a. Rat X2. Thymectomy; accessory thymus. Killed after 151 days. Upper incisor, convex side, about middle of tooth. Defective calcification of dentine, ingrowth of blood vessels from odontoblast layer. D3 = incompletely calcified dentine. C = capillary.

Fig. 2b. Concave side of same tooth. Same designations.

Fig. 2c. Lower incisor, concave side.

Fig. 2d. Lower incisor, convex side.

Plate 23.

Fig. 3a. Rat X5, control. Killed at same time as X2. Upper incisor, convex side.

Fig. 3b. Upper incisor, concave side. Deposition of calcium in layers.

Fig. 3c. Lower incisor, convex side.

Fig. 3d. Lower incisor, concave side. Alternating striæ of calcium-containing and calcium-free dentine.

Fig. 4a. Rat Y8(3). Injected subcutaneously with suspension of bone marrow of rachitic rat Y8. Killed after 28 days. Upper incisor. Incomplete calcification of dentine. Irregular disposition of odontoblasts. I = islands of uncalcified dentine. P = pulp.

Fig. 4b. Upper incisor, concave side. Dentine wholly calcium-free. Odontoblast layer indistinct.

FIG. 4c. Lower incisor, convex side.

FIG. 4d. Lower incisor, concave side. Dentine entirely uncalcified and convoluted. Ingrowth of odontoblasts and capillaries.

PLATE 24.

FIG. 5. Rat 50. Spontaneous rachitis. View of right half of thorax and vertebral column.

PLATE 25.

FIG. 6. Rat 50. Section of rib, low power drawing. K = zone of proliferating cartilage. K1 and K2 = islands of cartilage. O1 = osteoid tissue. O2 = calcified bone. P = ingrowth of periosteum. V = thickening of periosteum (old callus?). M = medullary cavity.

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BACTERIAL ANTIFERMENTS.

STUDIES ON FERMENT ACTION. XVII.*[1]

BY JAMES W. JOBLING, M.D., AND WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The resistance of bacteria to enzyme action, because of its importance as a factor in the defense of the invading organism against the destructive agencies of the host, has interested several observers, among them Kruse (1), Fermi (2), Kantorowicz (3), and Weinkopff (4). Influenced possibly by the epochal advances in protein chemistry and by the fact that bacterial antigens are probably purely protein in character, workers in immunology have naturally investigated with great care the bacterial proteins, and the effect of proteolytic enzymes upon them.

The older conception that bacteria resisted digestion because of some vital property had to be discarded when it was found that organisms killed by chloroform, toluol, carbolic acid, etc., seemed to resist digestion as well as viable bacteria. It has so far been the implied idea that since bacteria contain protein, such protein should be an available substrate for enzyme action, as casein or edestin might be used; in other words, that the bacterial cell represented a naked and a freely exposed mass of protein. In dealing with an intact cell there are, however, certain factors which would indicate that this position is untenable.

Were we to assume that bacteria have a limiting membrane, such a membrane would, if an analogy to plant cells is permissible, contain no protein (Czapek (5)); if similar to animal cells they would probably be protected by a predominatingly lipoidal membrane (Meyer, Overton). There is, of course, no direct evidence, although with the exception of Hamm (6) there has been a uniformity in the reports of the absence of nitrogenous material even from the capsular material derived from the lower organisms. The absence of cellulose in bacteria is of no great import, for many of the true fungi have no cellulose wall and are protected

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[1] We are under obligations to Dr. William H. Park and his associates, of the New York Board of Health, and to Dr. A. P. Hitchens, of Glenolden, Pa., who have kindly placed large amounts of diphtheria and tubercle bacilli at our disposal; and to Professor Hans Zinsser for numerous courtesies.

only by a delicate membrane; unfortunately the evidence as to the presence or absence of chitin, which might have some bearing on the subject, is conflicting.

Bacteria do, however, contain fats and lipoids in varying amounts, which, because of their marked effect on surface tension, would for purely physical reasons tend to become concentrated at the periphery of a colloidal system such as the bacterial protoplasm. With or without a morphologically distinct limiting membrane we can reasonably assume that the external surface of the bacterial cell is potentially lipoidal. Upon such a substrate we should expect lipolytic rather than proteolytic action. Immunologists have recognized that in cytolysis there are other factors than such distinctly protein manifestations of immunity as are exemplified by the precipitin and agglutinin reactions. Zinsser (7) has recently emphasized this fact. In this connection it is interesting to recall again an observation of Metchnikoff (8). Metchnikoff noted that the intestinal tract of the larva of certain insects (moths) was free from bacteria, and reasoned that this absence of intestinal flora must have some connection with the nature of the enzymes secreted into the gastro-intestinal tract. He calls attention to the fact that these insects, utilizing waxes and fats to a large extent, must secrete powerful lipases.

Oppenheimer (9), reviewing the subject of bacteriolysis from an entirely different standpoint, reaches a similar conclusion. He says: "I have formerly classified these substances (cytolysins) among the ferments because the view was prevalent that the destruction of the cell membrane was due to a specific proteolytic ferment." This view must be discarded, but it is probable that we may later place them among the lipases (10). In a recent paper (11) we have shown by microchemical analysis that bacteriolysis is not necessarily associated with proteolysis.

As briefly outlined, the failure of proteolysis by the action of proteolytic ferments on the intact cell offers then no problem in itself, for there is probably no exposed protein substrate upon which the enzyme may act.

Kantorowicz (3) has probably studied the subject most carefully. He confirmed the older observations of the absence of digestion of the intact cell whether living or dead, and showed that heating Gram-negative organisms to 70° C. made them lose their power of resistance, while Gram-positive organisms would resist digestion even after boiling. He concluded that the resistance was due to an antiferment. He found that dried organisms were as resistant as fresh bacteria, but noted that after drying, grinding in a mortar, and extracting with acetone, the organisms lost their resistance. He assumed two factors to be concerned in bacteriolysis, one of which had to do with the overcoming of the antiferment. Kruse (1) did not, however, agree with the conclusion of his pupil, and doubted that an antiferment as such was involved in the resistance of the organisms. He inclined to the view that differences in resistance among bacteria were due to differences in permeability of the organisms.

Fermi (2) working with cultures, introducing ferments into culture media, reached conclusions similar to those of Kantorowicz. He noted, however, that dried organisms were less resistant than those freshly killed.

The foregoing observations were made by using suspensions of organisms either living or killed in various ways, to which the enzyme was added and the resulting digestive effect observed by the relative clearing of the suspensions. This method is open to several objections. The interpretation of the results is inexact and arbitrary, depending on the observer's judgment, and varying under different conditions. Probably of more importance is the fact that solution of the organisms is interpreted as proteolysis. Solution of a bacterial cell may be largely a physical process related most intimately to changes in its lipoidal constituents. The actual proteolytic cleavage is a purely chemical phenomenon and cannot be determined by ocular observation. This distinction, to which Jobling and Strouse (10) have previously called attention, should be clearly kept in mind, for the careless use of these terms has led to considerable confusion in the interpretation of some immunological experiments. There is, furthermore, a technical error in that it is not possible by the means so far employed to present an approximately equal amount of substrate for the enzyme action, which is important when the comparative rate of digestion of different organisms is sought.

In order to obtain results which would be free from the objections above referred to, our observations have been based exclusively on the determination of non-coagulable nitrogen before and after digestion. These were made by means of the Folin method. Only by such a method can we determine the exact percentage of digestion. We have used organisms grown on agar in large flat bottles. The bacteria were washed, dried *in vacuo* at a low temperature, and then ground in an agate mortar. During the process of drying changes must occur in the colloidal state of the external limiting membrane, so that the uniform resistance of various bacteria to proteolytic action while intact is overcome, and the degree of resistance remaining should be an index of the actual antiferment power of the different organisms.

One other physical factor, the intimacy of the lipoid-protein combination, must be taken into consideration. Numerous workers have pointed out that the lipoids occurring in cells are so closely combined with the proteins that it is impossible by the ordinary

extraction methods to break up their union. This can be accomplished only by a complete hydrolysis of the protein. Thus the determination of bacterial lipoids by means of extraction in the Soxhlet apparatus will give results which are much too low, the real ratio of the lipoidal substances being obtained by extraction after saponification. We have noticed during the course of the work that the lipoids from the Gram-positive bacteria are proportionately extracted more slowly by means of lipoid solvents than from the Gram-negative organisms.

The bacterial emulsions were, as a rule, made up in a 1 per cent. suspension. When fresh organisms were used an effort was made to have the bacterial concentration about one milligram of nitrogen per cubic centimeter. The total non-coagulable nitrogen both before and after digestion was determined by precipitating the coagulable protein with acetic acid and salt, boiling for ten minutes, filtering through kaolinized hard filter paper, and making the usual Folin determination on the filtrate. The trypsin used was freshly prepared before each experiment by dissolving a dried preparation in physiological salt solution.

The lipoid content of the bacteria was determined by saponifying on the water-bath for two hours with alcoholic potash, acidifying, and extracting thoroughly with ether. The iodin value of the lipoids was obtained by the usual Wijs method. It should be noted, however, that the iodin determinations, while often repeated, were of necessity carried out on very small amounts of lipoids, and some caution must be observed in basing conclusions on single observations. When it was not possible to repeat the determinations, we have placed a question mark with the figure to denote the fact. Further observations which we hope to make on larger amounts of material will no doubt give more accurate values. It is reasonable to assume that the lipoid extract and the degree of unsaturation will show marked fluctuations under the varying conditions of growth, nutrition, age, and oxygenation, and, as will be noted, the variations in the total lipoid content of different growths of the same organism were at times marked. Lyons (12) has studied the influence of sugars on the fat content of bacteria, and Cramer (13) has carried out similar experiments.

The amount of lipoids (including fats) has been determined by several observers, as shown in the following table (table I). Inasmuch as the majority of these observations were made by simple ether extraction, the values given are in some instances probably much too low.

TABLE I.

Organism.	Lipoids (including fats).	Observer.
Tubercle bacilli	36–44 per cent.	Baudran (14).
Tubercle bacilli	40 per cent.	Kresling (15).
Tubercle bacilli	31.56 per cent.	Levene (16).
Tubercle bacilli	37.57 per cent.	de Schweinitz and Dorset (17).
Tubercle bacilli	26.2–28 per cent.	Hammerschlag (18).
Bacillus mallei	7.91 per cent.	de Schweinitz and Dorset (19).
Diphtheria bacillus	1.62 per cent.	Dzierzgowski and Rekowski (20).
Meningococci	5.94 per cent.	Ditthorn and Woerner (21).
Pneumobacillus	1.7 per cent.	Brieger (22).
Anthrax bacillus	7.8 per cent.	Dyrmont (23).
Bacillus prodigiosus	4.83 per cent.	Kappes (24).
Bacillus xerosis	8.06 per cent.	Kappes (24).

PROTOCOL I.

Effect of Lipoidal Extraction on the Rate of Digestion of Tubercle Bacilli.

Bacterial suspension 1 c.c.	Trypsin.	Total nitrogen.	Gross digestion.	Digestion, per cent.	Lipoid content, per cent.
Tubercle bacilli[2] (dried)	0.2 c.c.	0.55 mg.	0.13	23	31.2
Tubercle bacilli (extracted with ether, chloroform, or alcohol)	0.2 c.c.	0.74 mg.	0.33	44	9
Tubercle bacilli (extracted in Soxhlet apparatus) (ether 120 hrs.; alcohol 100 hrs.; benzol 50 hrs.)	0.2 c.c.	0.80 mg.	0.46	57	7

The suspensions were made up with an equal amount of sodium carbonate solution.

To these we can add the following determinations:

Tubercle bacilli	32.7 per cent.
Staphylococci	4.51–8.5 per cent.
Diphtheria bacilli	5.5 –7.5 per cent.
Typhoid bacilli	7.0 –8.2 per cent.
Colon bacilli	4.2 –8.15 per cent.
Subtilis bacilli	1.7 per cent.

There are no available determinations of the iodin values of

[2] Previously killed by boiling.

these lipoids with the exception of those made upon the waxes and fats of the tubercle bacillus.

The values which we have found are as follows:

Tubercle bacilli	20 for total lipoids; 24.2 for fatty acids.
Staphylococci	60–80
Diphtheria bacilli	80–110
Typhoid bacilli	33–38
Colon bacilli	32–40
Subtilis bacilli	44 (?)
Tetanus bacilli	44 (?)

Having previously shown that the unsaturated fatty acid radical is antitryptic and its inhibition proportional to the degree of unsaturation (25); that such unsaturated lipoids can be isolated from tubercle bacilli (26) and from tuberculous caseous material (27); that serum antitrypsin consists of similar lipoids, either as free fatty acids, as esters of cholesterol, or combined with lecithin (28), we considered it reasonable to assume that bacteria might resist digestion in a degree proportional to the amount of the unsaturated lipoids contained. It will be remembered that Kantorowicz found that the antiferment was removed from the bacteria when they were ground up and extracted with acetone.

INFLUENCE OF LIPOIDAL EXTRACTION ON DIGESTIBILITY.

We first determined the influence of lipoidal extraction on the tryptic digestion of tubercle bacilli. 1 per cent. suspensions of (1) freshly dried organisms, of (2) extracted bacilli (with 9 per cent. of lipoids), and of (3) extracted bacilli (with 7 per cent. of lipoids) were used. The rate of digestion is shown in protocol I. The organisms used in this work were obtained from Dr. Hitchens and had been killed by heating at high temperatures. In addition they were old, and so some of the unsaturated fatty acids may have become oxidized.

A similar result was obtained with other organisms, as is shown in the following experiment with typhoid and colon bacilli (protocol II). Müller (29) noted that typhoid bacilli from which the lipoids had been extracted were more easily digested by leucocytes.

PROTOCOL II.

Effect of Lipoidal Extraction on the Rate of Digestion of Colon and Typhoid Bacilli.

Bacterial suspensions 1 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent.
Colon bacilli	1.77 mg.	0.17 mg.	1.60 mg.	0.545 (−0.17)	0.375 mg.	23
Ether-extracted colon bacilli	1.87 mg.	0.23 mg.	1.64 mg.	0.95 (−0.23)	0.72 mg.	44
Typhoid bacilli	1.08 mg.	0.14 mg.	0.94 mg.	0.66 (−0.14)	0.52 mg.	56
Ether-extracted typhoid bacilli	1.0 mg.	0.2 mg.	0.8 mg.	0.87 (−0.2)	0.67 mg.	84

THE LIPOIDS AS ANTIFERMENTS.

We have previously noted that the lipoids extracted from tubercle bacilli were antitryptic when saponified (26). That the same is true of the lipoids from other organisms is shown in the following experiment.

Pure cultures of staphylococci and of colon bacilli were removed from bottles, washed, and saponified. After acidification, the lipoids were extracted and again saponified. The soaps were then dissolved in physiological salt solution. Various amounts were mixed with one unit of trypsin, incubated for thirty minutes, and casein was then added for digestion. In another set of tubes the unsaponified bacterial lipoids, suspended in salt solution, were used in equal amounts. The relative inhibition will be noted in protocol III.

PROTOCOL III.

Effect of Bacterial Lipoids, Saponified and Emulsified, on Tryptic Digestion.

Tube No.	Casein.	Trypsin.	Staphylococci lipoids saponified.	Colon lipoids saponified.	Staphylococci lipoids emulsified.	Colon lipoids emulsified.	Gross casein digestion.	Digestion, per cent.
1	2 c.c.	0	0	0	0	0	0.0 mg.	0
2	2 c.c.	0.1 c.c.	0	0	0	0	1.66 mg.	100
3	2 c.c.	0.1 c.c.	2 c.c.	0	0	0	0.217 mg.	13
4	2 c.c.	0.1 c.c.	0	2 c.c.	0	0	0.715 mg.	43
5	2 c.c.	0.1 c.c.	0	0	2 c.c.	0	1.33 mg.	80
6	2 c.c.	0.1 c.c.	0	0	0	2 c.c.	1.54 mg.	92

The iodin value of the lipoids was 97 for those from the staphylococci, and 32 from the colon bacilli.

THE COMPARATIVE RATE OF DIGESTION.

We next studied the comparative rate of digestion of bacteria, and the relation to the lipoids contained in the bacteria. Such an experiment is shown in protocol IV, and has for convenience been charted (text-figure 1). As a lipoidal antitryptic index we may multiply the percentage of lipoids by the iodin value, as determined for the organisms used in this experiment.

PROTOCOL IV.

Comparative Rate of Tryptic Digestion of Bacteria.

Bacterial suspension 1 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent
Diphtheria bacilli.....	1 0 mg.	0.12 mg.	0.88 mg.	0.28(−0.12)	0.16 mg.	18
Staphylococci.....	0.83 mg.	0.15 mg.	0.68 mg.	0.31(−0.15)	0.16 mg.	23
Typhoid bacilli.....	1.42 mg.	0.21 mg.	1.21 mg	0.5 (−0.21)	0.29 mg.	24
Typhoid bacilli (ether-extracted).....	0.9 mg.	0.35 mg.	0.55 mg.	0.66(−0.35)	0.31 mg.	56
Tubercle bacilli (ether-extracted).....	0.83 mg.	0.12 mg.	0.71 mg.	0.4 (−0.12)	0.28 mg.	40
Colon bacilli.....	0.8 mg.	0.12 mg.	0.68 mg.	0.39(−0.12)	0.27 mg.	40

Diphtheria bacilli ..... 7 per cent. × 80 = 560
Staphylococci ..... 8 per cent. × 60 = 480
Typhoid bacilli ..... 8 per cent. × 30 = 240
Typhoid bacilli (extracted) ..... ? ? ?
Colon bacilli ..... 4 per cent. × 40 = 160
Tubercle bacilli (extracted) ..... 7 per cent. × 20 = 140

This offers, of course, only a general approximation, and cannot take into consideration the fact that the lipoids vary no doubt in the intimacy of their connection with the proteins, a factor which must influence their ability to protect the protein. The digestion as determined was as follows:

Diphtheria bacilli ..... 18 per cent.
Staphylococci ..... 23 per cent.
Typhoid bacilli ..... 24 per cent.
Typhoid bacilli (extracted) ..... 56 per cent.
Colon bacilli ..... 40 per cent.
Tubercle bacilli (extracted) ..... 40 per cent.

TEXT-FIG. 1. Relation of the antiferment index to the rate of digestion of various organisms.

These figures show a striking parallelism between the antitryptic property and the lipoidal protecting bodies.

A similar experiment can be shown if we compare the rate of digestion of the Gram-positive organisms, the lipoids being as follows:

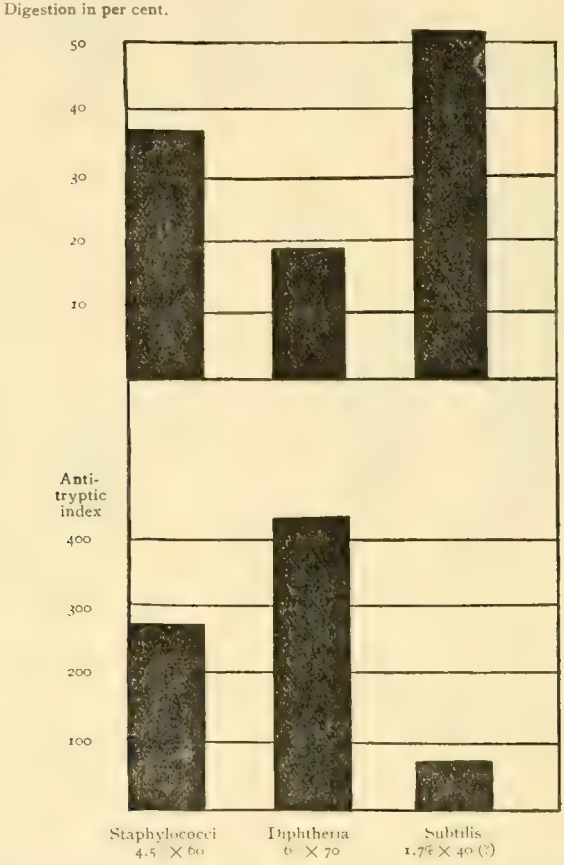
Diphtheria bacilli ..... 6.0 per cent. × 70 = 420
Staphylococci ..... 4.5 per cent. × 60 = 270
Subtilis bacilli ..... 1.7 per cent. × 40 = 68

PROTOCOL V.

Comparative Rate of Digestion of Staphylococci and Diphtheria and Subtilis Bacilli.

Bacterial suspension 2 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent.
Staphylococci.....	1.54 mg.	0.375 mg.	1.17 mg.	0.8 (−0.375)	0.425	36
Diphtheria bacilli.....	1.66 mg.	0.19 mg.	1.47 mg.	0.47 (−0.19)	0.27	19
Subtilis bacilli.....	1.3 mg.	0.48 mg.	0.82 mg.	0.909 (−0.48)	0.429	52

The organisms digested in the order indicated, the digestion being least with the diphtheria bacilli. The results are shown in protocol V and in text-figure 2.



TEXT-FIG. 2. Relation of the antiferment index to the rate of digestion of Gram-positive organisms.

THE EFFECT OF HEAT.

It has been mentioned that a difference existed in the resistance to heat of the Gram-positive and the Gram-negative organisms. This is shown in the following experiments.

A suspension of typhoid bacilli was heated at 50°, 60°, 70°, and 100° C. The

tubes were then subjected to the action of trypsin. The percentage of digestion was as follows:

50° C. = 31 per cent.
60° C. = 31 per cent.
70° C. = 36 per cent.
100° C. = 41 per cent.

As Kantorowicz (3) has found, there is a reduction of the resistance after heating to 70° C., but the effect is not so striking as one might expect if a complete destruction of the antiferment had resulted. The Gram-positive organisms, staphylococci for example, show no change after heating, unless it is a slight increase in the resistance. The percentage of digestion was as follows:

Unheated = 55 per cent.
50° C. = 54 per cent.
60° C. = 35 per cent.
70° C. = 41 per cent.
100° C. = 38 per cent.

In a second experiment the results were as follows:

Unheated organisms = 16 per cent.
70° C. = 18 per cent.
100° C. = 14 per cent.

LIPOIDAL ADSORPTION BY BACTERIA.

We have previously mentioned that bacteria suspended in normal serum are able to adsorb the unsaturated lipoids from the serum, and by virtue thereof to become more resistant to the action of trypsin (30). Such an experiment follows.

A suspension of typhoid bacilli was incubated with 10 c.c. of normal guinea pig serum for six hours. The bacteria were then centrifuged, washed, and dried at a low temperature. Untreated bacteria were used as a control. Emulsions were prepared containing 1 mg. of nitrogen per cubic centimeter, and to each was added 0.1 c.c. of trypsin solution and 2 c.c. of a 0.5 per cent. solution of sodium carbonate. Digestion was permitted for six hours. The digestion of the untreated bacteria was 75 per cent., of the serum-treated bacteria 23 per cent.

The lipoidal adsorption can be shown in a more direct manner, as is illustrated in the following experiment.

Dried subtilis bacilli were added to 25 c.c. of horse serum and incubated for three hours. The bacteria were then washed after being centrifuged from the serum. The lipoid content of the bacteria and of the supernatant serum was now determined.

Total lipoids in 25 c.c. of original serum	0.083	gm.
Total lipoids in 25 c.c. after incubating with bacteria	0.060	gm.
	0.023	gm. loss.
Weight of bacteria recovered 0.6 gm.		
Lipoid content originally present, 1.7 per cent. 0.0102 gm.		
Total lipoids recovered from bacteria	0.036	gm.
Less lipoids originally present	0.0102	gm.
	0.0258	gm. gain.

While there has been a loss of 0.023 of a gram in the ether-soluble substances from the serum, the bacteria show a corresponding gain. The absorption of lipoids is made especially evident in this case because of the small lipoid content of the organisms used. The lipoid determinations were made after thorough saponification of both bacteria and serum on the water-bath for three hours.

DISCUSSION.

Inasmuch as the bacterial lipoids may show more marked fluctuations than the other constituents, the quantity depending largely on the composition of the nutrient media and to some extent on the age of the culture, and the chemical quality, unsaturation, being subject to changing oxidative conditions, we are of the opinion that the demonstration that the antiferment property of bacteria depends on definite quantitative as well as qualitative differences in the lipoidal constituents of the bacteria may offer the means of solution of some still obscure problems in immunity. Thus in the study of virulence, depending on the invasive power of the organisms and on their resistance against the defensive mechanism of the host a partial solution might be found in a comparative study of the lipoids under different conditions of bacterial existence. We have experiments under way to elucidate this point. So too, a further study of the adsorption of the lipoids from the serum offers an attractive field. While in this process the resistance of the intact bacteria to proteolytic enzymes is not increased, for on such organisms the

enzymes probably find no substrate upon which they may become fixed and active, nevertheless the metabolic processes of the cell, depending wholly upon the rate of exchange of nutritive and excretory products through the cell membrane, must be profoundly altered. Indeed it might be conceived that such a disturbance of the proper functioning of the membrane would under some conditions lead to the death of the cell. On the other hand, an absorption of serum lipoids, by preventing the excretion of toxic metabolic products, such as leucocydins, virulins, etc., might bring about phagocytosis of organisms otherwise resistant. That the so called opsonins are closely related to the lipoids seems to have been established by the work of Stuber (31).

That the bacterial lipoids influence the intracellular proteolytic activity becomes apparent when we compare the amount of non-coagulable nitrogen contained in various organisms. Thus diphtheria bacilli, with a high antiferment index, have only from 5 to 7 per cent. of the nitrogenous material in a non-coagulable form, whereas in subtilis bacilli, with a low antiferment index, a correspondingly greater protease activity is indicated by the presence of from 33 to 35 per cent. of the total nitrogen in a non-coagulable form. Staphylococci, typhoid bacilli, and colon bacilli occupy an intermediate position. This influence is analogous to the effect of the antiferment on the protein metabolism of higher organisms, as we shall show in a subsequent paper.

The antiferment, being non-specific, is able not only to inhibit the action of the digestive ferments, but is probably the agent concerned in preventing excessive protease action, or autolysis, in the cells. It is easy to understand that with the death of the cells and subsequent development of an acidity because of the failure in the removal of acid waste products, the antiferment property is greatly lessened by changes in the state of dispersion brought about by the increase in acidity. This lowering of the antiferment property of the unsaturated lipoids by a change in their dispersion is most easily demonstrated with serum antitrypsin. The morphological expression of the process is possibly to be found in the development of the so called myelin figures found in cells stained during the early stages of autolysis. It is probable that the increase in the rate of

autolysis following the addition of certain inorganic colloids to liver emulsions, noted by Ascoli and Izar (32), depends on an absorption of the lipoidal antiferments.

Kantorowicz (3), in view of the difference in behavior of the antiferment in Gram-negative and Gram-positive organisms, is inclined to the view that the antiferments are in this case different. It seems more reasonable to assume that the difference noted is due to a purely physical condition. It is possible that in the Gram-positive organisms the lipoids are in more intimate association with the protein molecule, so that when dispersion changes are brought about, as by heating, the relative protection by these lipoids remained unaltered. This idea would find support in view of the fact that the lipoids from Gram-positive organisms resist extraction by lipoid solvents to a greater extent than do those from Gram-negative bacteria.

In view of the work of Tamura (33), definitely showing that the bacterial constituent responsible for the Gram stain is lipoidal in that it can be found in the lipoid extract, and as the unsaturated fatty acids and their salts take the Gram stain easily, because the iodin can be bound to the unsaturated carbon atoms, it would seem rational to seek some connection between the degree of unsaturation of the bacterial lipoids and the Gram staining ability. From this point of view the high iodin values of the lipoids from diphtheria bacilli and staphylococci, as compared with those of typhoid or colon bacilli, might seem significant, were it not for the fact that in the case of the tubercle and subtilis bacilli, the results are apparently the reverse. Knudson (34), working in Gies's laboratory, finds, however, that the lipoids from tubercle bacilli, which have a low iodin value (24), contain several of the higher unsaturated fatty acids.

As a result of our study we are inclined to emphasize the importance of the unsaturated lipoids as representing probably a most important factor in the regulation of the proteolytic activity of the cellular metabolism, due to the fact that the protein lipoid combination resists the action of the proteolytic enzymes.

CONCLUSIONS.

1. Intact bacteria probably resist tryptic digestion because of the absence of an exposed protein substrate.

2. Dried organisms resist digestion in a degree proportional to their content of unsaturated lipoids.

3. Lipoidal extractives reduce the resistance to tryptic digestion.

4. The extracted lipoids (saponified) are antitryptic in a degree proportional to their unsaturation.

5. The inactivation of the antiferment in Gram-negative organisms is probably due to changes in the degree of lipoidal dispersion.

6. Bacteria adsorb lipoids from the serum when incubated at 37° C. Such organisms when dried are found to be more resistant to tryptic digestion than untreated organisms.

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PERIARTERITIS NODOSA—A CLINICAL AND PATHOLOGICAL REVIEW OF THE DISEASE

WITH A REPORT OF TWO CASES *

ALBERT R. LAMB, M.D.
NEW YORK

INTRODUCTION

A number of diseases which are to-day not uncommon and which are recognized clinically without much difficulty were at one time quite rare, owing largely to the fact that so little was known about them that they either escaped notice or were incorrectly diagnosed. It is quite possible that the rather rare but exceedingly interesting affection known as periarteritis nodosa is a case in point. Most of the cases have been observed in Germany, where the disease was first recognized and where the clinicians and pathologists have come to be familiar with it, whereas in this country, where the disease has attracted very little attention, only three cases have been reported up to the present time.[1]

Within the past year at the Presbyterian Hospital, we have had the exceptional opportunity of seeing two cases. Including these, the total number of reported cases now numbers but thirty-eight. Most of the reports have concerned themselves largely with the pathologic histology of the disease and, except for some minor differences of opinion, this aspect of the affection has been well covered. We are still quite in the dark as to the cause, however, and many of the histories are too brief to aid greatly in arriving at a correct idea of the symptom-complex. Indeed, many observers are inclined to the belief that, barring the presence of the subcutaneous nodules, which are present in only a small minority of the cases, there is no set of symptoms on which a diagnosis can be made. One cannot escape the conviction, however, that an increasing familiarity with the disease may lead to some means of diagnosis whereby more cases may be recognized and that the chance for study during life thus afforded may clear up the present obscure etiology. It, therefore, seems most desirable that all cases of periarteritis nodosa be reported as completely as possible. And, in view of the extreme rarity of the disease in this country, the present seems an opportune time for a complete review of the known facts concerning it.

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* From the Pathological Department, Presbyterian Hospital, Columbia University, New York.

1. The two cases here given were very briefly reported at the New York Pathological Society during the past year.

REPORT OF TWO CASES

For permission to use the clinical records of these two cases I am greatly indebted to Drs. Janeway, Bovaird and Lambert on whose services in the hospital they occurred.

Case 1.—Man, aged 26, mechanic, ill about seven weeks, was admitted to the hospital, Dec. 7, 1912. The family history was unimportant.

Past History.—The patient had typhoid at the age of 4. Two years ago he had an attack of grip. Venereal disease is denied. He does not use tobacco; takes an occasional glass of beer.

Present History.—For the past two years patient has had asthma. At first the attacks came about once a month, lately as often as two or three in a week. About three weeks before admission he noticed intense itching about the right ankle and in eight or ten hours an oval, node-like swelling the size of an egg appeared. The overlying skin was tense, shiny and covered with bright red macules measuring about 4 mm. in diameter. The swelling gradually lessened and the macules underwent the color graduations of a contusion. In two or three days the swelling and eruption had entirely disappeared. Pain and tenderness were severe at the height of the swelling, disappearing with its subsidence. The night after the swelling appeared he had a severe asthmatic attack lasting all night. Expectoration was quite profuse and contained clotted blood. Since then he has had a purpuric eruption appearing on different parts of the body roughly every other night with asthmatic attacks on the alternate nights. All parts of his body have been affected except the face. For the past two weeks, accompanying the rash, he has had severe abdominal cramps lasting for ten or twelve hours and ceasing after vomiting. Vomitus consisted of a thin green liquid with a few blood-clots. At times, with the appearance of the eruption he would have pains in various joints and these would be swollen, slightly red, tender and very painful on any motion. Bowels moved once or twice a day and were normal as far as he knows. Appetite is good; no loss of weight; no fever or night-sweats. For the past two weeks patient has been up ten or twelve times at nights to urinate.

Physical Examination.—Pale and sallow. Superficial lymph-nodes slightly enlarged. Eyes normal. Gums firm. No throat lesions. Lungs: Moderate relative dulness at right apex posteriorly; feeble inspiratory murmur with prolonged expiration and high-pitched piping râles over all. Heart: Apex in fifth space, 10.5 cm. to left of mid line; sounds normal, no murmurs. Arteries small, soft and just palpable. Blood-pressure 145-105. Abdomen rather full and tympanitic; liver not felt; edge of spleen just felt at costal margin on deep inspiration; no masses or rigidity. Both legs show numerous small hemorrhagic areas in various stages of involution. These areas vary in size from petechiae to irregular confluent areas about 1 cm. in length. The larger ones are grouped about the ankles and are deep wine color. The lesions are somewhat raised, firm and dry with a tendency to scaling. On the left thigh, just above the knee, there is a large, somewhat elevated, firm area the size of the last joint of the thumb. It is pale green, somewhat tender and apparently in the course of the internal saphenous vein but not connected with it. On the right thigh there is an elongated, raised area measuring 3 by 1 cm. It is reddish brown with dark red spots in the center, is very slightly tender and for its whole length lies over a superficial vein. About the knees there are a few pale green discolorations. Over the lower end of the right tibia there is an irregular depressed ulcer. It is dry and the floor is made up of a deep red blood-clot. Below the left patella there is some thickening in the region of the bursa, with slight pain on flexion of the leg. The most probable diagnosis seemed to be Schönlein's "purpura rheumatica."

Course.—The temperature on admission was 100.4 F., gradually falling to normal on the fifth day. It remained normal for three days and then rose

gradually to 101.8. In a few days it was again normal and then subnormal for five days. There was another short rise to 100.4, followed by another normal period and a terminal rise to 102. The pulse varied between 85 and 130 and was generally more rapid than one would expect for the temperature. When the temperature was subnormal, the pulse was between 80 and 110.

Urine.—Eight examinations. The specific gravity varied between 1.010 and 1.018. There was always a trace of albumin present and on five occasions it was estimated at from 0.15 to 0.22 per cent. No sugar was found. There were always hyaline and granular casts present and at times epithelial and waxy casts as well; also a moderate number of leukocytes and a few to many red blood-cells.

The blood-counts showed the following result:

Date	R. B. C. No.	Hb. Pct.	W. B. C. No.	Polys. Pct.	Lymphos. Pct.	L. Monos. Pct.	Eosin. Pct.
December 9	.....	75	17,200	50	14	2	34
December 10	3,500,000	65	20,200	51	11	0	38
December 12	.....	..	.....	32	12	5	51
December 31	3,384,000	42	20,900	58	17	2	23

December 9: Sputum shows many red blood-cells and ciliated epithelial cells but no Charcot-Leyden crystals, Laennec's pearls or Curschmann's spirals.

December 12: Eye-grounds negative.

December 14: Wassermann reaction (Noguchi modification) negative.

December 17: Since yesterday afternoon patient has complained of constant, sharp abdominal pain without any other change in the physical signs. No vomiting. Stools normal. No tubercle bacilli found in the sputum.

December 18: Phthalein test: Color appeared in twelve minutes. Amount at the end of two hours too small to estimate. Sputum bloody. Coughs less. Feels better. Abdominal pain has disappeared. Lips and nails quite cyanotic. No tubercle bacilli in sputum.

December 20: Slight pain in the shoulders and neck this morning. Has a considerable amount of yellowish-green sputum with considerable fresh blood. The lungs show nothing more than the signs of asthma and bronchitis. No pain in the chest. Considerable relief from epinephrin.

December 29: White, rather glistening lines are seen along the vessels of the left retina as they come through the disk.

December 31: Gastric contents: Total acidity, 38. Free hydrochloric acid, 6. No lactic acid. Moderate reaction for blood. No ova or parasites in the stool. A second phthalein test showed the color in seventeen minutes, but the amount at the end of two hours was again too small to estimate.

January 1: The veins and glands in both inguinal regions are very sore and tender. In the left femoral region there is a small, red, tender area.

January 2: There is more redness and swelling in the left femoral region. On the back of the left leg there is a slightly swollen, reddened area measuring about 4 by 6 cm. The right hypochondrium is distinctly tender and resistant.

Jnuary 5: There is a pericardial friction-rub present this morning and a pleural friction-rub at the left base. Lumbar puncture: Twenty c.c. of clear fluid obtained. Cultures sterile. Urea, 0.323 per cent. Sodium chlorid, 0.812.

January 6: Died to-day after increasing stupor for two days. Gradual failure of pulse and respiration before death. During the last sixty hours voided only 1,000 c.c. of urine. The blood-culture taken in two-broth flasks, January 3, remained sterile. Blood-pressure varied between 135 and 165. The urine quantity varied between 690 and 1,920 c.c. On thirteen days it was below 1,000 c.c.

Post-Mortem Examination.—The diagnosis of periarteritis nodosa was not suspected at the time of the necropsy. When the microscopic sections were examined several days later and the characteristic lesion found in the kidney, the organs from the patient were again carefully examined; but the only sug-

gestion of arterial lesions was found in the heart, where there were one or two pin-head-sized whitish nodules on one of the small coronary branches. No nodules were found in any of the other organs. Unfortunately, none of the subcutaneous tissue in the regions of the eruption and swellings was examined.

The essential points in the necropsy were the following:

The body is strikingly pale and considerably emaciated. There are ecchymoses about the right knee and about both elbow-joints. The peritoneal cavity contains a small amount of cloudy yellow fluid, and the intestines show a scattered fibrinopurulent exudate. The lungs are adherent to the chest-wall and to the outer surface of the pericardium by fresh fibrinous adhesions.

Lungs: Both are edematous and show scattered areas of bronchopneumonia. The bronchi are intensely congested and contain mucopurulent material. The vessels appear normal.

Bronchial Lymph-Nodes: Moderately enlarged, deeply pigmented and firm.

Heart: The pericardium contains about 50 c.c. of cloudy yellow fluid; there are several fresh fibrinous adhesions stretching between the two surfaces and the surfaces are covered with a shaggy, fibrinopurulent exudate and show scattered petechial hemorrhages. The heart is not enlarged. There is a small, firm, irregular nodule at the base of one of the pulmonic cusps and the mitral cusps show small, irregularly nodular, reddish vegetations, the largest about the size of a small pea. The other valves and the heart chambers show no change. In general the heart-muscle is homogeneous and dull reddish-brown, but there are scattered areas of a more dirty yellow color. The main portions of the coronary arteries show no sclerosis. The aorta is smooth and elastic throughout its course.

Spleen: Measures 12 by 7 by 3 cm. There is perisplenitis. Otherwise the organ appears normal.

Liver: Measures 29 by 19 by 6 cm. There is a small area of superficial atrophy in the right lobe. On section, the liver shows no abnormal change.

Gall-Bladder: Normal. Ducts patent.

Kidneys: Left measures 12 by 7 by 3.5 cm. The capsule strips easily, exposing a smooth yellowish-pink surface with a rather diffuse mottling of yellow. On section the cortex does not average more than 5 mm. in thickness and is slightly less than this in some places. The striations are indistinct and quite distorted. The glomeruli are quite distinct as colorless dots. The pelvis and ureter are normal.

Right: Measures 11.5 by 6 by 3.5 cm. and is quite similar in all respects to the left.

Adrenals and esophagus appear normal.

Stomach: Contains a little brownish fluid and a moderate amount of mucus. There is considerable injection of the mucous membrane and a few small petechial hemorrhages.

Pancreas and Ducts: Normal.

Mesenteric Lymph-Nodes: These are enlarged, many to bean-size. Several of them are broken down and ooze creamy-yellow pus on section.

Intestines: Show nothing more than a few areas of congestion in the lower part of the ileum.

Bladder and Prostate: Normal.

Brain and Spinal Cord: Permission could not be obtained for their examination.

Microscopic Examination.—Lungs: Many sections from various parts of the lungs were examined. These show a thickened pleura, extensive pulmonary edema with congestion of the alveolar walls and swelling of the alveolar epithelium and scattered areas of hemorrhagic bronchopneumonia. There is some increase of connective tissue about the bronchi together with numerous mononuclear wandering cells and eosinophils. Scattered here and there in the alveoli are small clumps of Gram-positive cocci. The large vessels appear quite

normal and in most of the sections even the smaller arteries show no change; but one does find a few small arteries both about the bronchi and in the lung-tissue proper which are not normal. The changes consist in an adventitial and periarterial infiltration with mononuclear wandering cells and eosinophils, polymorphonuclear leukocytes being almost entirely absent. The changes are generally unilateral or at least more marked on one side, and the media and intima are normal. An occasional small artery is seen, however, whose lumen is almost entirely occluded. These changes apparently represent an early stage of the lesion seen in the arteries in other organs.

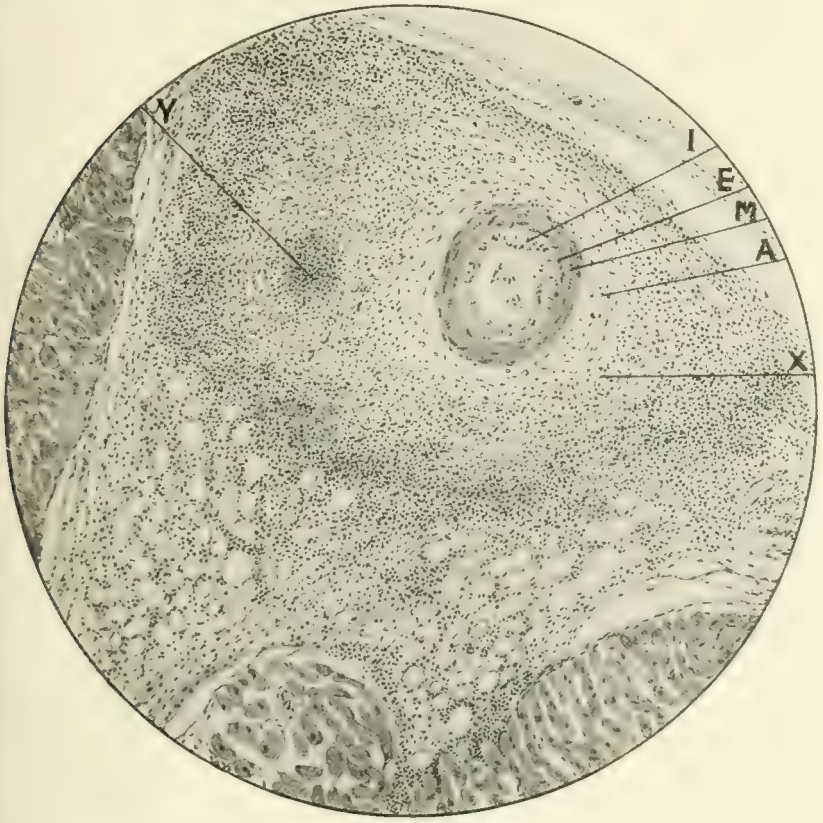

Fig. 1.—Case 1. Section of a small coronary branch just beneath the epicardium showing very pronounced periarterial infiltration with a small area of beginning necrosis on one side. The media, while still intact, takes the eosin stain rather strongly. *I*, intima; *E*, internal elastica; *M*, media; *A*, adventitia; *X*, periarterial infiltration; *Y*, area of beginning necrosis.

Bronchial Lymph-Node: In the capsule, two small arteries are found showing early changes similar to those seen in the lung. The arteries in the gland itself show no change. There is considrable pigmentation and hyperplasia.

Vegetation of Mitral Valve: Consists of a very vascular and quite cellular mass of connective tissue richly invaded by mononuclear wandering cells. On the surface is an irregular thrombus mass containing clumps of bacteria. These appear to be cocci. Smears from these vegetations show a few pairs of cocci surrounded by a small space and also some rather fat Gram-positive bacilli.

Heart: The pericardium shows an acute inflammatory exudate. There is cloudy swelling of the muscle-fibers and patchy interstitial myocarditis about the vessels. A section of one of the papillary muscles shows a most extensive fibrosis with large areas of granulation tissue and wandering-cell infiltration. Most of the vessels show no change. Some of the small arteries, while showing no changes in their coats, have a little surrounding cellular infiltration, the cells being generally mononuclear wandering cells and eosinophils. One section, taken through one of the tiny macroscopic nodules, shows the periarterial character of the lesion very well (Figure 1). It affects a small artery just beneath the pericardium. The lumen is narrowed, being encroached on by the moderately proliferated intima, and contains red blood-cells, among which are some fibroblasts extending in from the intima. The intima is thicker on one side, its texture quite loose and its cells irregular in size and shape. Some of

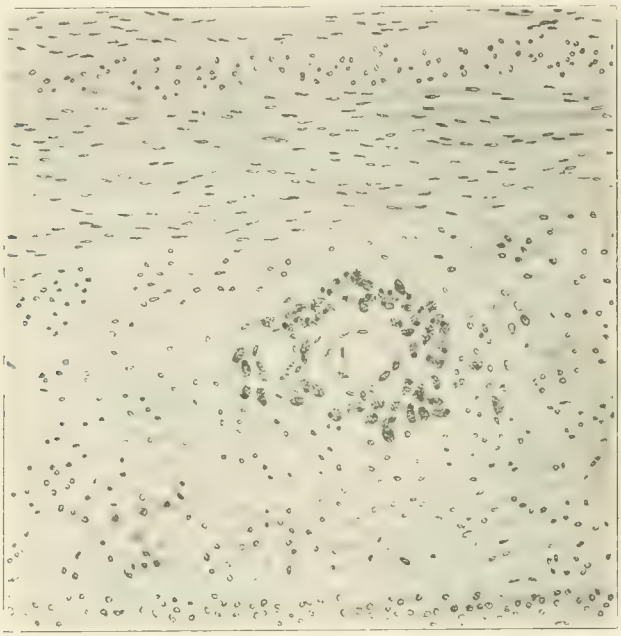

Fig. 2.—Case 1. Section of a small artery in the muscular coat of the stomach. Represents a very early lesion. The intima and media are normal. The elastic lamellae are not changed. The adventitia is loose in texture and is densely infiltrated with eosinophils.

them are quite definitely fibroblasts; others look like distorted endothelial cells. The internal elastica is well preserved except at one point, where it is wanting opposite a small necrotic area in the media. The media appears granular and the nuclei indistinct but it shows no cellular infiltration. There is no characteristic hyaline ring, but the media gives one the impressioon of approaching toward such a condition as seen in other vessels. The fibers appear more granular and take the eosin stain more intensely. The adventitia is much thickened and merges imperceptibly into the tremendous periarterial cellular infiltration and granulation tissue. The cells are wandering cells, fibroblasts and a most striking number of eosinophils. Many of them are plasma-cells. While polymorphonuclear leukocytes are scarce, one does find some. All of these cells infiltrate the surrounding fat for a considerable distance. At one side there is a small area of necrosis.

Spleen: Eosinophils are rather strikingly numerous and seem especially attracted to the vessels. The small arteries are somewhat thickened but none of them shows any characteristic change.

Liver: Shows cloudy swelling and one small area of superficial atrophy. Some of the portal spaces show a little increase of connective tissue with a few wandering cells. There are no distinct arterial changes.

Pancreas: Shows no change except for a little wandering-cell infiltration about one or two of the small arteries. Mixed with these cells are a few eosinophils.

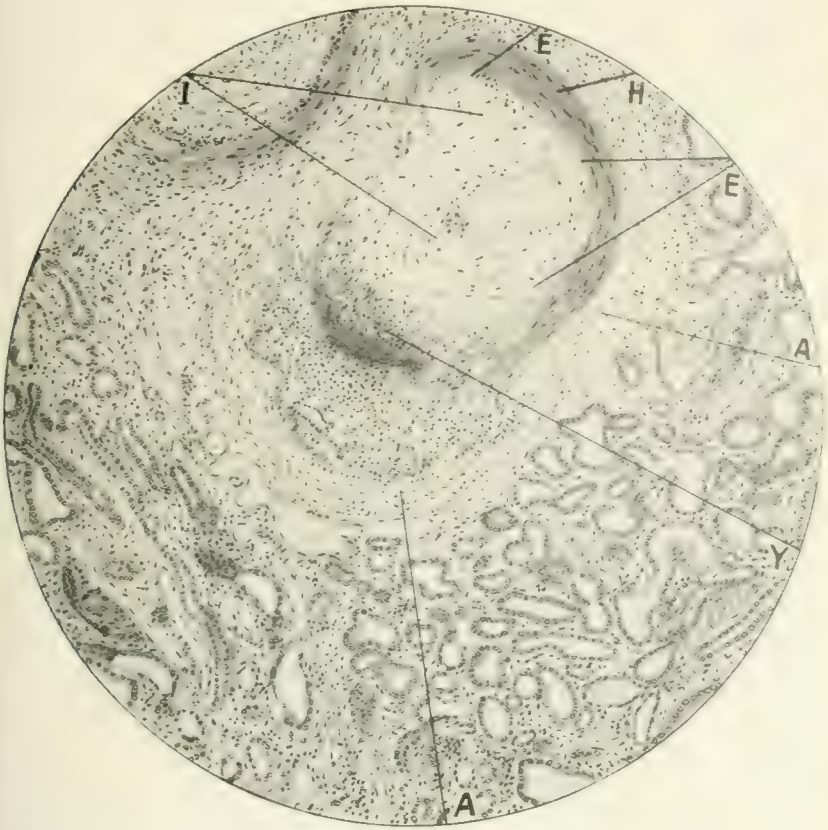

Fig. 3.—Case 1. Section of artery in kidney. The intima, *I*, is much thickened and is surrounded for two-thirds of the circumference by the internal elastic lamella which does not show in drawing. Just external to this is the hyaline ring, *H*, which fuses with the area of necrosis at *Y*. Adventitia, *A*, apparently extends outside the area of necrosis. *E*, internal elastica as shown in other sections stained with elastic-tissue stain. It is wanting opposite the area of necrosis.

Mesenteric Glands: Many small arteries are seen both within the gland and in the surrounding tissue which show early changes.

Stomach: The various coats of the organ appear normal. In one section, in the muscular coat, there is a cross-section of a small artery (Figure 2) which is of interest as it possibly shows a very early lesion. The intima and media appear normal and the elastic lamellae are unchanged. The adventitia is loose

in texture and cannot be differentiated from the adjacent tissue. It is densely infiltrated with eosinophils and a few cells with large, oval, vesicular nuclei. No further change is made out in tracing the vessel through serial sections. While one cannot be certain that this represents a very early change, the eosinophils which are found so abundantly in the lesions elsewhere seem to point to this conclusion. Another section shows a typical lesion of a small artery in

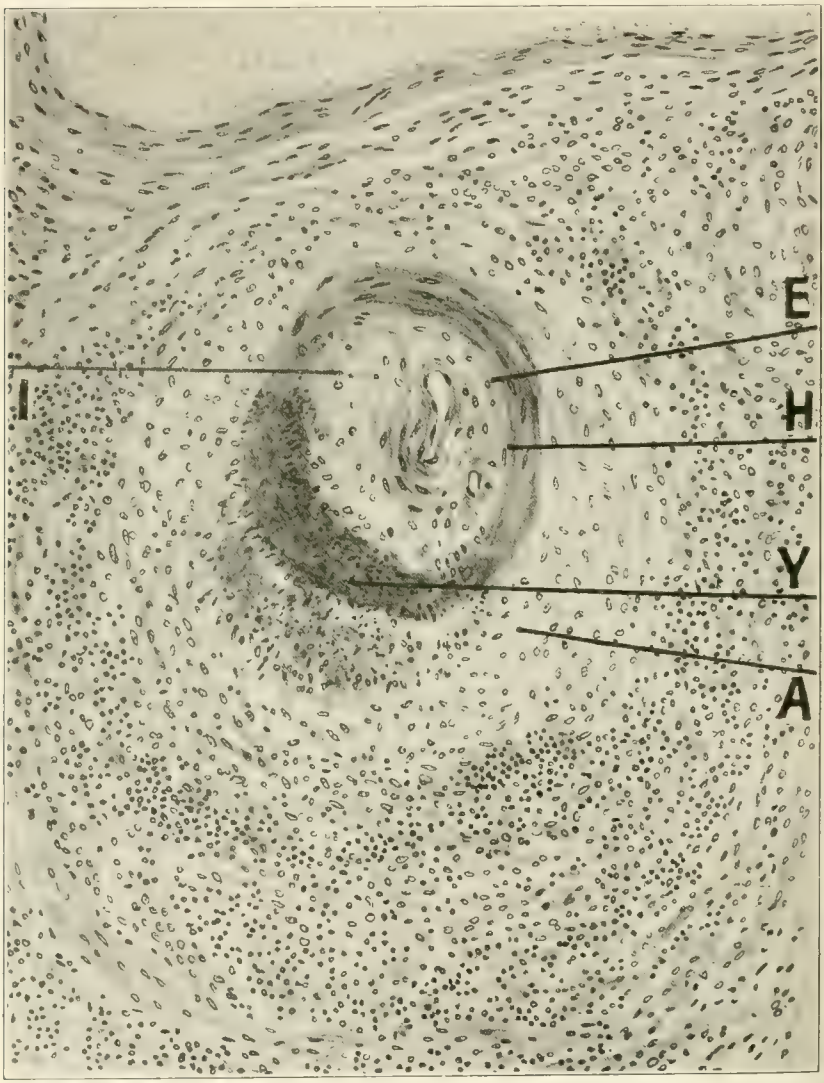

Fig. 4.—Case 1. Section of artery in adrenal. The intima, *I*, is thickened. The internal elastica, *E*, is intact except in the region of necrosis, *Y*, where it has disappeared. The hyaline ring, *H*, in which one can still make out the cells of the media lies just external to the elastica and fuses with the area of necrosis at *Y*. Adventitia, A, merges imperceptibly with the periarterial infiltration.

the submucosa. An elastic-tissue stain shows the internal elastica ending abruptly after encircling about one-half the circumference while the external elastica is completely gone. Another vessel shows similar changes but with an added complete thrombosis.

Intestines: There is an acute inflammatory peritoneal exudate, intense congestion of the vessels and some hemorrhages in the mucosa. A number of the small arteries show typical early changes and two are completely thrombosed.

Kidneys: These show the most striking changes of any of the organs, and it was here that the lesion was first noticed. In this place (Figure 3) the lumen of the artery is small and the intima very much thickened, being composed of a loose connective tissue, rich in fibroblasts and containing a few eosinophils and nuclear fragments. Surrounding the intima for about two-thirds of the circumference, the internal elastica is intact but swollen in places. Just outside of it and limited by it, is the characteristic, intensely eosin-stained, hyaline ring in which one can still make out the cells of the media. It gives the impression of being a more advanced stage of the appearance seen in the section of a coronary artery. At one side, the elastica has disappeared, the hyaline ring melts into an area of necrosis and the cellular infiltration of the adventitia breaks through into the intima. The adventitia is composed of young connective tissue with some wandering-cell infiltration and at this point seems to extend outside the area of necrosis which is mainly in the media. While this is the most advanced lesion seen in any of the many kidney-sections, many of the small arteries show similar if less marked changes. There is an exquisite acute, subacute and chronic glomerulonephritis, the glomeruli showing all stages of adherence to the capsules with epithelial crescents and hyaline thrombi. There is an extensive diffuse increase of connective tissue throughout and a striking wandering-cell infiltration with many eosinophils in the interstitial tissue. The vessels are all congested and many of the tubules contain blood. Some of the tubules are practically obliterated while some are dilated and cyst-like.

Adrenals: The arteries of the capsule show the lesion beautifully, the hyaline ring being especially well marked (Figure 4). As in the kidney, this ring is situated just outside the internal elastica which is preserved for about two-thirds of the circumference and then melts into an area of intensely eosin-stained necrosis in the media. The intima is loose in texture and rather loculated. The cells are large and contain oval, vesicular nuclei. There is an intense periarterial exudate and granulation tissue with many eosinophils. The gland itself shows no change.

Bacteriologic Findings at Necropsy.—Heart-blood: Two broth-flasks show colon bacilli and Gram-positive cocci in chains. The latter were overgrown by the former and were not isolated.

Peritoneal Exudate: Smears show pus-cells and many chain cocci. No other bacteria. Cultures give a pure growth of streptococci.

Pericardial Fluid: Smears show unformed detritus, rarely a pus-cell. No bacteria found. Cultures in broth and ascitic glucose-agar are sterile.

Mesenteric Lymph-Node: Smears show cocci in chains and many pus-cells. Cultures not taken. Unfortunately the cultural characteristics of the streptococcus isolated from this case were not worked out so that it is impossible to compare it with the organism obtained in the second case.

Careful examination of many sections stained for bacteria by various methods failed to show any in the vascular lesions in spite of the fact that the necropsy cultures showed a bacteremia. No spirochetes could be found in sections stained by the Levaditi method.

Case 2.—Girl, aged 10, ill five weeks, was admitted to the hospital Jan. 18, 1914. The family history was negative.

Past History.—Five years ago patient had a slight attack of pain in the epigastrium somewhat similar to the present attack but less severe. It lasted only one day. Had measles as a baby and diphtheria five years ago.

Present History.—Nine days before admission patient was taken suddenly ill in the night with severe pain in the epigastrium. Felt better the next day and was up and about for two days, when the pain came on again and she vomited twice. The pain was sharp and well localized to a small area high up in the midline. Since that time she has been in bed and has had the pain intermittently but always in the same place; has felt feverish, had a headache and has vomited several times. Her throat was sore but has been better for the last few days. She says that she had blood in her sputum at times. No pain is felt on micturition and no blood appears in the urine.

Physical Examination.—Patient well developed and large for her age. Appears acutely ill. No eruption present.

Pharynx: Reddened and shows a considerable thin mucoid, blood-tinged exudate. The left tonsil is enlarged, the right slightly so.

Tongue: Dry and heavily coated.

Lymph-Nodes: The anterior cervical and axillary are palpable.

Heart: Is not enlarged, sounds regular and of good force. No murmurs. Pulses equal and regular. Low tension. Walls not palpable.

Lungs: Clear except for a few transient râles at the left base.

Liver and Spleen: Not palpable.

Extremities: Reflexes normal. No edema.

Abdomen: There is general abdominal fulness and resistance which seems involuntary. No true rigidity. There is tenderness over the whole lower abdomen, more marked on the right side. No dulness in the flanks. No masses. The temperature on admission was 102.6 F. and the blood-count showed 33,100 leukocytes with 93 per cent. of polymorphonuclear leukocytes. A diagnosis of appendicitis was made and she was operated on shortly after entering the hospital. The appendix and peritoneal cavity were found to be normal. The abdomen was explored but no cause found for the symptoms.

January 20: Blood-culture, two broth-flasks sterile.

January 21: There is tenderness and evidence of fluid in both knee-joints and tenderness in both hip-joints. Heart normal.

January 22: There is tenderness in the left shoulder-joint. Temperature between 102 and 103. Wassermann reaction, three plus.

January 23: Shoulder still painful. Signs in the knees have cleared up. Heart normal. Chill.

January 27: Right knee shows a small amount of fluid. Slight amount of edema in the region of the Achilles tendon of both legs. The abdomen is distended in its upper half but not tender. No dulness in the flanks. Spleen not palpable. Heart and lungs normal. There is a little edema of the dependent part of the back.

January 28: Throat culture shows a predominant streptococcus. Patient seen by Dr. Janeway, who believes that she is suffering either from sepsis following a streptococcus sore throat and has a toxic nephritis or that she has nephritis following an unrecognized scarlet fever. Transferred to the medical service. During her stay on the surgical side the urine showed a specific gravity of between 1.012 and 1.028. Albumin, from a very faint trace to a trace. A few to many hyaline and granular casts. No red blood-cells. A blood-count taken January 19 showed 26,200 leukocytes with 92 per cent. of polymorphonuclears, and one taken January 28 showed practically the same. The temperature varied between 101 and 103.6 for a week. It was then lower reaching normal January 27 and on the following two days was between 99 and 100.4. The pulse corresponded with the temperature, varying between 74 and 128. The surgical diagnosis was acute tonsillitis, acute nephritis and acute toxic arthritis. She was apathetic at times, quite thirsty and vomited repeatedly.

On admission to the medical ward her physical examination was unchanged. There was tenderness over the whole abdomen, most marked in the midline

above the umbilicus. Very slight edema of the back. The right wrist was somewhat stiff and painful on motion. The right shoulder was somewhat painful.

January 29: White blood-cells, 17,000; polymorphonuclears, 95 per cent.; lymphocytes 5 per cent.

February 1: Liver felt 2 inches below the costal margin. White blood-cells, 52,000; polymorphonuclears, 95 per cent.; lymphocytes, 4 per cent.; large mononuclears, 1 per cent.

February 2: Blood-culture, two broth-flasks sterile. Patient is quite drowsy. There is a mottled erythema over the upper part of her forearms.

February 3: White blood-corpuscles, 66,000; polymorphonuclears, 95 per cent.; lymphocytes, 5 per cent. There is a systolic murmur at the apex. The eruption has disappeared. General condition improved. The right wrist is swollen and painful on motion.

February 5: White blood-corpuscles, 39,800; polymorphonuclears, 90 per cent.; lymphocytes, 9 per cent.; large mononuclears, 1 per cent. Wassermann reaction negative. Blood-culture, two ascitic glucose-agar plates, sterile.

February 6: Metacarpophalangeal joints of both hands swollen and tender. Right wrist better. There is a dusky mottling of the face, whole trunk and extremities, especially over the back and shoulders. It is in the nature of a diffuse erythema.

February 7: Vomitus, 175 c.c. of thick yellow fluid. Total acidity 24, no free hydrochloric acid. Faint reaction for lactic acid. Blood-count: White blood-corpuscles, 40,400; polymorphonuclears, 73 per cent.; lymphocytes, 27 per cent.

February 10: Temperature is lower and joints have cleared up. For the past three days has suffered from paroxysms of abdominal pain and complains of pain on the slightest pressure. There is no rigidity. Rectal examination negative.

February 11: White blood-corpuscles, 26,600; polymorphonuclears, 88 per cent.; lymphocytes, 12 per cent. Vomitus, 155 c.c. of a dark brownish fluid. Faint reaction for blood. Total acidity 8, free hydrochloric acid 4. No lactic acid. Stool: Yellow, fluid; contains small flecks of mucus; moderate reaction for blood; urobilin, moderate. Microscopic examination: Mucus, red cells, pus-cells and epithelial cells. No ova or parasites.

February 14: Patient died. For the preceding week was very apathetic and disliked to be moved or examined. Lay on her side with legs drawn up. At times severe abdominal pains were present which generally required morphin for relief. The pain seemed to be more in the region of the umbilicus. There was tenderness or hyperesthesia over the whole abdomen, but no resistance or rigidity. No signs of fluid. Vomited two or three times a day, generally after taking food. On the day of death, suddenly awoke early in the afternoon, cried out on account of severe abdominal pain, attempted to throw off the bedclothes, became very cyanotic and had a very rapid, almost imperceptible pulse. Relieved by morphin and went to sleep. About three hours later she cried out again, gave a few gasping respirations and died.

Her temperature varied between 99 and 103.2, during the last week between 99 and 101.5. Pulse corresponded, in rate, ranging between 74 and 120. Blood-pressure, from 104 to 60. Urine: Twelve examinations showed the specific gravity quite constant between 1.012 and 1.018, with from a trace to a very faint trace of albumin, generally a very faint trace. Most of the examinations showed hyaline and granular casts. The amount of urine varied between 150 and 1,600 c.c., being 1.000 c.c. or over on only three occasions.

Post-Mortem Examination.—Two and one-half hours post mortem. The body is that of a strikingly pale, moderately wasted young girl. There is a little puffiness of the eyelids and slight edema of the ankles. No subcutaneous nodules found. The abdomen is moderately distended. None of the joints

appears swollen. The abdomen contains about 300 c.c. of clear amber fluid. The peritoneal surfaces are smooth and glistening except for a few small shreds of fibrin over the intestinal coils. The appendix is missing, the omentum being adherent to the stump. No nodules seen on any of the vessels. On opening the thorax, the lungs are found partially collapsed and retracted, exposing a large precordial surface. There is about 100 c.c. of clear fluid in each pleural cavity and the same amount in the pericardial sac. The diagnosis was immediately apparent on exposing the heart, the coronary branches showing numerous characteristic nodules (Fig. 5). The heart weighs 320 gm.

Fig. 5.—Case 2. Heart showing thickly set nodules on coronary branches with overlying petechial hemorrhages.

Right Coronary: At the origin of the infundibular branch there is a globular swelling measuring 6 mm. in diameter, filled with a grayish-red adherent thrombus. Near the termination of this branch there is a small yellowish-white nodule of about 2 mm. in diameter. The transverse branch is almost occluded by connective tissue but shows no nodules. The marginal branch shows a small aneurysmal dilatation, partially filled with thrombus just after it leaves the main artery. In the rest of its course and in its branches are numerous, often closely set, oval and globular, firm swellings, the largest measuring about 3 mm. in diameter. On section, these appear as partially occluded vessels with a very thick whitish periphery. The descending branch shows no external change.

Left Coronary: The main stem is free. The first portion of the descending branch is quite tortuous and contains a thrombus. A little further on there is a bluish-red aneurysm measuring 1 by 0.5 cm. and showing a few petechial spots over it. It is filled with a thrombus. Near the apex there are a few firm nodular swellings. The transverse branch shows similar nodules and in the branches

Fig. 6.—Case 2. External view of kidney showing numerous dark raised areas due to hemorrhagic infarcts separated by sunken grayish tissue. The main renal artery and branches show no nodules.

which it gives off over the anterior and posterior surfaces of the ventricles there are numerous firm nodules, the largest measuring 2 or 3 mm. in diameter. Over these, discrete and conglomerate petechial hemorrhages are numerous. A section through any portion of the heart-wall shows the arteries very much

thickened, the lumen generally completely occluded or represented by a minute reddish dot and the adjacent muscle showing irregular yellowish opaque areas. The veins are not changed. The valves and the various chambers of the heart are all quite normal. The aorta and large branches show no change.

Lungs: Except for a moderate collapse, the lungs appear quite normal and one cannot make out any changes in the vessels or bronchi.

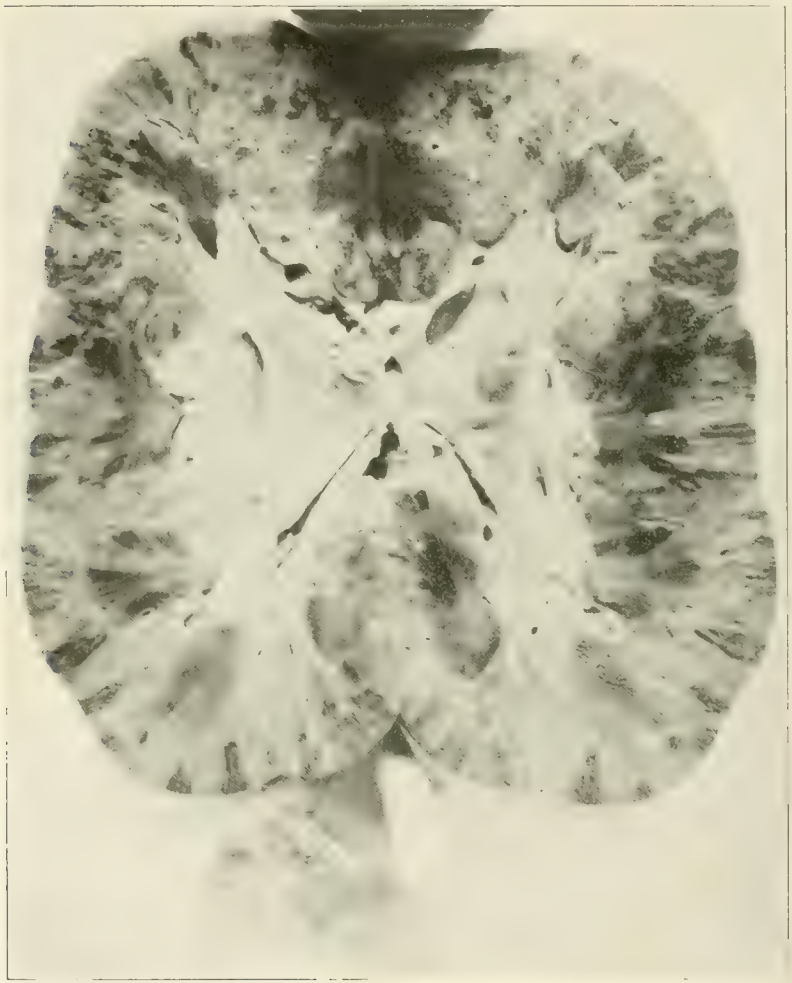

Fig. 7.—Case 2. Same kidney as Fig. 6, seen on section. Shows the infarcts distinctly. The numerous little nodules along the small arteries can also be made out.

Liver: Weighs 1,400 gm. The surface is smooth and brownish yellow with irregular areas of congestion. On section, the tissue about the portal vessels appears a little increased in amount and the vessels a little thickened. No definite nodules seen.

Gall-Bladder: Contains viscid greenish bile. The mucosa appears normal and the ducts are patent.

Spleen: Weighs 160 gm. The capsule is tense but smooth and the organ is quite firm. The cut surface remains quite flat and the pulp is firm. The color is purplish red and the malpighian corpuscles are unusually numerous and larger than normal. One obtains the impression that the small central arteries are slightly thickened.

Pancreas: Is large and of normal consistency. On section, it shows nothing abnormal. No macroscopic lesions of the arteries can be seen.

Stomach: Appears quite normal. No lesion of any of the vessels.

The esophagus, trachea, larynx, thyroid and parathyroids appear normal.

Kidneys: Are quite alike (Figs. 6 and 7). The left weighs 160 gm. and the right 150 gm. The fatty capsule is moderately thick but strips easily. The true capsule is easily removed without tearing the renal surface which has a most remarkable appearance. It is coarsely irregular and in color, a mottled red and opaque yellowish gray with a sprinkling of small yellowish points. The raised portions are a deep red with small gray dots. They are abundant, in the aggregate representing fully one-half of the surface, are quite irregular in outline, several often running together to form map-like patterns and they are separated by slightly sunken, dull, opaque, yellowish-gray tissue containing flecks of yellow. The same appearance is seen on section. The cortex averages from 6 to 8 mm. in thickness. The vessels of the pyramids are much congested. The cortex and tissue between the pyramids has a rather rough, furred appearance and one can make out numerous, small, hard pearly gray nodules along the course of the vessels. These resemble miliary tubercles. There are many, larger and smaller, deep red and more grayish-red, necrotic-looking, roughly wedge-shaped areas extending from the surface deeply into the cortex and in places into the medulla. These correspond with the raised areas on the surfaces. They are separated by slightly sunken, yellowish-gray opaque tissue in which are tiny nodules and flecks of yellow. The main renal arteries and their larger branches show no change. An attempt was made to inject the kidney but no gelatin could be forced through the vessels. The kidneys were almost exactly like the colored illustration in Dickson's article.

Adrenals: These are of normal size. On section there are several small vessels which seem thickened and show thrombi.

Uterus: Undeveloped. The tubes, vagina and ovaries all appear normal.

Intestines: Duodenum normal. In the ileum, there are two small, widely separated areas which show a little hemorrhage of the crests of the rugae. In the cecum, there is a small cavity at the appendix stump filled with reddish, grumous material. Otherwise the intestines show no change and the vessels are quite normal in appearance.

Omentum and mesentery are normal.

Mesenteric lymph-nodes: Moderately enlarged and show some thickened vessels with thrombi but no definite nodules.

Arteries: The celiac axis and its branches show no change. In fact, none of the larger arteries shows anything abnormal. The veins are quite normal. The blood in all the vessels is quite fluid. Permission could not be obtained to examine the nervous system, joints or extremities.

Microscopic Examination.—Heart: In one section, taken at a distance from the region of the nodules, the muscle appears fairly normal. The striations are distinct and the vessels quite normal but congested. Around one small vessel there is a little infiltration with wandering cells penetrating between the muscle fibers. Among these cells there are a few eosinophils. In another section there are several irregular areas of fibroblasts replacing the muscle-fibers. A section through the largest aneurysmal nodule shows a fair sized, dilated vessel completely filled with an adherent thrombus which shows an invasion of fibroblasts from the vessel-wall. The wall is much changed. Only in one small area is the internal elastica present and here it is in direct relation with the thrombus. Immediately external to it there is a ring of intensely eosin-stained material in

which nuclei are still seen. In places, the media appears rarefied and shows spaces, in other regions the vessel-wall is composed of fibrous granulation tissue. The adjacent heart-muscle shows granular degeneration and irregular fibrous-tissue replacement. Near the aneurysm there is a smaller vessel seen in cross-section. One can make out a delicate endothelial lining, external to which there is a very loose tissue, internal elastica, homogeneous hyaline ring and a marked adventitial infiltration with mononuclear wandering cells and fibroblasts, among them an occasional eosinophil. The adjacent heart-muscle is granular and degenerated and shows considerable fibrous tissue. Another vessel is seen in which the infiltration is largely perivascular. A final heart-section shows a large artery filled with thrombus showing beginning organization about the periphery. The heart-muscle is cloudy and shows scattered wandering cells and considerable fibrous tissue. In the artery, the elastica is quite gone. The homogeneous hyaline ring is quite distinct close to the intima and at one point it splits into two layers enclosing a granulation tissue containing beautiful fibroblasts and hemorrhage. The perivascular tissue is very cellular, showing fibroblasts, wandering cells and many eosinophils. A longitudinal section of a small vessel shows the coats normal but a considerable similar cellular exudate in the perivascular tissue. Eosinophils are numerous. A cross-section of another vessel shows the lumen filled with beautiful granulation tissue and in another there is canalization of the thrombus.

Aorta: Appears normal. Several small branches at the side of the aorta show characteristic changes of a rather late type.

Lungs: The lung-tissue shows slight collapse with some thickening of the alveolar walls. Sections taken near the surface show no vascular lesion either in the pulmonary or bronchial arteries. Sections from the hilum show very pretty lesions of the bronchial arteries but none of the pulmonics. There is a small lymph-gland present in which eosinophils are very numerous.

Bronchial Glands: The follicles are moderately well shown. The sinuses are dilated and contain relatively few cells. One or two small arteries are seen which show the changes seen elsewhere, eosinophils being quite numerous. Some of the follicles show hyaline degeneration.

Spleen: This is much congested, the blood-spaces showing up very clearly. In some of the malpighian corpuscles there is hyaline degeneration of the central cells. In one section, there is an irregular area of infarction. Eosinophils are quite numerous throughout. Several of the small arteries show characteristic changes. In some, the lesion appears as an adventitial cellular infiltration, with a rather marked invasion of the media with wandering cells and fibroblasts, the muscle being almost entirely replaced. In one or two of the small arteries of the malpighian corpuscles there is a fringe of cells just outside the adventitia, most of which are eosinophils.

Liver: The cells show cloudy swelling and in many places vacuoles. In most of the portal spaces there is an infiltration of wandering cells and eosinophils, often quite definitely about the arteries. In a few of the arteries the lesion is quite definite. Another section so taken as to give a longitudinal view of the vessels and bile-ducts gives a very good picture of the lesion and shows considerable surrounding fibrosis.

Pancreas: The lesions here are exquisite. In general, the parenchymal cells and islands are normal, but there is a moderate increase in the fibrous tissue between the lobules. In one region, there is a definite area of necrosis, apparently an infarct, the supplying arteries being severely changed. The striking thing in the section is the lesion of the vessels. Almost every artery shows most extensive changes and there is a very marked perivascular wandering-cell infiltration with fibrosis. One of the prettiest lesions in the whole case is seen in a longitudinal section of a small artery (Fig. 8). At one end the artery is perfectly normal. It then shows a wider and more irregular lumen with a moderately thickened intima in which are various-sized irregular nuclei, an internal elastica which is more or less preserved until one reaches the region

of most extensive change where it gradually fades out, a homogeneous hyaline band on either side of the vessel which is in places internal to the elastica, in other places external and for some distance divided by it. External to this one cannot differentiate between media and adventitia. One comes on a loose mass of various-sized wandering cells and eosinophils with a few fibroblasts and outside of these a young connective tissue with numerous eosinophils. In serial sections, the lesion can be traced into normal artery once more.

Esophagus: The lesion is seen in one or two of the small arteries of the serosa.

Stomach: Normal. One small artery is seen in the muscularis with a little cellular infiltration about it.

Colon: A longitudinal section of a small vessel shows it normal but on either side of its wall the branches show extensive changes.

Ileum: Normal.

Cecum: One vessel shows the lesion. External to the cecum there is an area of granulation tissue the borders of which are necrotic. This represents the softened area at the base of the appendix described in the gross. Appendix is normal.

Mesenteric Lymph-Nodes: Appear much the same as the bronchial.

Lymph-Node at Gall-Bladder: Shows various areas of hyaline degeneration but no vascular lesions.

Thyroid: Normal. Vessels unaffected.

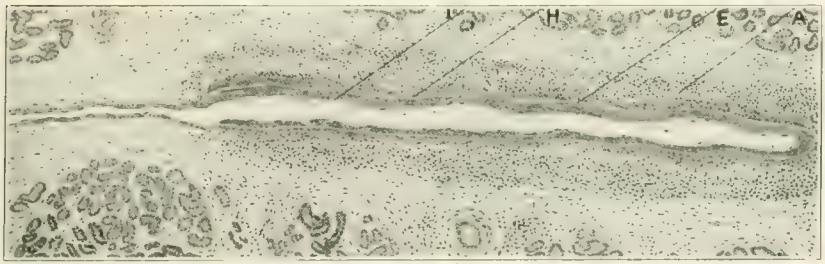

Fig. 8.—Case 2. Longitudinal section of artery in pancreas. The rather abrupt beginning of the lesion is well seen at the left. The intima, *I*, is thickened and is indistinguishable from the hyaline ring, *H*. The internal elastica, *E*, thus apparently divides this hyaline ring. The media is represented by the hyaline ring and the adventitia, *A*, merges with the periarterial infiltration. In other sections the lesion can be traced to the right into normal artery again.

Kidneys: Sections show very numerous fresh hemorrhagic and older infarcts. A large portion of the cortex is thus affected and the infarcted areas often run down to and into the medulla. The small amount of intervening renal tissue is fairly normal except for the vascular changes which are very extensive, almost every small artery being affected. There is considerable perivascular infiltration. One fairly large artery shows an extensive thickening of the intima, the internal elastica remaining intact. The thickening consists of a bluish-pink material rich in spindle-shaped fibroblasts, many of the cells having peculiar little spaces about them. The media is composed of a similar tissue while the adventitia is more fibrous. The other vascular lesions are merely repetitions of the changes seen elsewhere.

Adrenals: Several of the small arteries about the organ show characteristic lesions. There are several infarcted areas in the cortex.

Fallopian Tube: One vessel found which shows typical changes.

Ovary: Normal.

Psoas Muscle: One small artery is seen which shows a beginning infiltration about the adventitia. The muscle-fibers are normal.

Pectoral Muscle: Very distinct changes are seen about many of the small arteries, the muscle-fibers remaining unaffected.

Uterus and Bladder: Normal.

Bacteriologic Findings at Necropsy.—Heart-Blood: Cultures were taken in various mediums, two ascitic glucose-agar shake-cultures, three ascitic glucose-agar slants and one neutral broth. These all remained perfectly sterile. At the end of three weeks they were examined, although there had been no sign of any growth. At present, three months after the necropsy, no growth has appeared.

Peritoneum: Cultures showed a *Staphylococcus aureus.*

Pericardial Fluid: One tube remained sterile, the other showed a colon bacillus. The most promising field for obtaining successful cultural results seemed to lie in the examination of the cardiac nodules and consequently most of the attention was directed to them. In the first place numerous smears were made from these nodules. These were stained by various methods but gave entirely negative results. The largest aneurysmal nodule was excised and washed in three changes of sterile salt solution. It was then divided into two portions, using Rosenow's sterile method. One portion was ground up in sterile salt solution. Of this, one guinea-pig received 0.5 c.c. intraperitoneally, one received 1 c.c. intraperitoneally and two rabbits received 0.5 and 1 c.c. respectively, intravenously. All of these animals remained perfectly well. They were finally killed, May. 1, two and one-half months after inoculation. None of them showed any lesions and the microscopic sections of the various organs failed to reveal any changes.

The second portion of the nodule was again divided and after flaming the pieces were planted at the bottom of two ascitic glucose-agar tubes. The rest of the bacteriologic work on the case was done by Dr. Coulter, and I am indebted to him for the following facts: After eighteen hours, both of the tubes were clouded. Direct examination and subcultures showed *Bacillus subtilis.* The nodules were at once removed, held within a flame until there was free spattering of fat and the entire surface was shriveled and discolored and then were replanted in ascitic glucose agar. The nodules sank to the bottom of the tube before the medium hardened. The ascitic fluid used in these cultures had been repeatedly tested for sterility. One tube has remained perfectly sterile. One tube showed no growth for two weeks.

On the sixteenth day there were two separate areas of clouding at the bottom of the tube, between the nodule and the glass. Each area was somewhat fan-shaped with the wide portion of the fan directed away from the nodule. Three days later there was very slight if any increase in the areas of clouding. A long sterile pipet was introduced to the bottom of the tube encountering considerable resistance owing to the firmness of the medium. At the very bottom, however, about the nodule, there were found several tenths of a cubic centimeter of slightly turbid fluid. About 0.1 c.c. of this fluid was inoculated into each of three ascitic glucose-agar cultures, one being a surface slant, the other two deep cultures similar to the one from which the fluid was withdrawn. Smears of this fluid showed rather large Gram-positive cocci, mostly in chains of from four to ten, but occasionally arranged as diplococci or tetrads. About the latter there was an indistinct capsule. On the following day the subcultures showed the following:

Surface cultures: Profuse growth, for the most part confluent with very numerous, uniformly distributed, more opaque areas. Growth emulsifies easily. It is not very viscid but strings out slightly when touched with the loop. The medium is not precipitated but the growth has extended between the medium and the glass. The medium was freshly slanted before inoculation so that it was quite moist.

Deep Cultures: Uniformly clouded throughout. No gas bubbles or cracks in the medium. Smears showed organism identical with those in the original culture. Smears from the surface growth showed the cocci smaller, somewhat

flattened and generally arranged as diplococci, chain formation being very slight. Plates were made from all of the cultures. In all there was but one type of colony in twenty-four hours. This was large, 1.5 by 2.5 mm. in diameter, moist, surface convex and glistening, outline smooth and regular, color uniformly grayish, consistency only slightly viscid.

Growth in peptone broth shows a uniform clouding with very slight sedimentation in six hours. In twenty-four hours there is marked sedimentation, but the fluid remains uniformly cloudy. Smears show chains of ten or more cocci with a distinct capsule. The organism is not bile-soluble and shows the following fermentation reactions: Dextrose +, dextrin +, saccharose +, lactose +, galactose +, inulin —, raffinose —, mannite —, salicin +, litmus milk +, acid, coagulated but not dissolved.

Animal inoculations were made from the second generation of deep cultures. An emulsion was made up to the strength of 500,000,000 to the cubic centimeter and of this a guinea-pig received 0.75 c.c. intraperitoneally, a rabbit 1.5 c.c. intravenously and a monkey 1.5 c.c. intravenously. None of these animals showed any reaction. They continued perfectly well and were killed two months later. They showed no gross or microscopic lesions. Another rabbit was given 5 c.c. of an emulsion containing 1,000,000,000 to the cubic centimeter. Death resulted in eighteen hours. The organism was recovered at the necropsy. There were no gross or microscopic lesions of the vessels, especially no signs of inflammation about the vessels or in the perivascular lymphatics.

Pieces of tissue were preserved in alcohol, formaldehyd and Zenker's fluid and sections were stained by various bacterial stains. A careful search was made of many sections for bacteria, without results. In some of the sections, especially those of the pancreas, there were some small bodies resembling cocci, but they could not be differentiated from the numerous particles of chromatin present. A set of sections was also stained by the Levaditi method but with entirely negative results.

The foregoing results have been reported in detail because they represent the most extensive bacteriologic investigations yet made on the disease. The examinations can be considered quite negative with the possible exception of the streptococcus isolated from the cardiac nodule. The occurrence of a *Staphylococcus aureus* in the peritoneal fluid seems to be merely a coincidence. Had it any real relation to the disease, it seems inconceivable that it should not have been obtained from the other cultures both during life and at the necropsy. As the cardiac nodule showed a contamination at the start it is, of course, quite possible that the streptococcus was one likewise. Against this is the fact that the nodule was very thoroughly seared and that the growth did not appear until a considerable period of time had elapsed. Even if this organism did come from the nodule it may have been there merely as an accidental secondary infection. As the occurrence of a streptococcus has been the only moderately constant finding in necropsy cultures of other cases it has seemed important to me to include the cultural characteristics of the one found in this case for the purpose of comparison in case any similar organism is isolated in the future.

The animal inoculations have proved nothing except that the disease was not transmitted to them either by the direct inoculation of material from the nodules or by the streptococcus isolated. It is quite possible

that the disease cannot be transmitted to these animals. It would have been interesting to have inoculated stags with material from the nodules, but unfortunately this was not possible.

CASES REPORTED UP TO THE PRESENT TIME

The following is a list of cases of periarteritis nodosa reported up to the present time:

Rokitansky, 1852. Studied microscopically by Eppinger thirty-five years later.
Kussmaul and Maier, 1866.
Zimmerman, 1874.
Chvostek and Weichselbaum, 1877.
P. Meyer, 1878.
Eppinger, 1887. Kundrat's case and microscopic description of Rokitansky's.
Fletcher, 1892. Necropsy by von Kahlden.
von Kahlden, 1894.
Graf, 1896.
Rosenblath, 1897.
Freund, 1899.
Krzyszkowski, 1899.
P. Müller, 1899. Two cases.
Schmorl, 1903.
Ferrari, 1903.
Veszprémi and Jancsó, 1903.
Schreiber, 1904.
Mönckeberg, 1905.
Versé, 1905.
Benedict, 1907.
Oberndorfer, 1907.
Bomhard, 1908. Same case as that reported by Oberndorfer.
Benda, 1908. Two cases.
Dickson, 1908.
J. E. Schmidt, 1908.
Longcope, 1908.
Hart, 1908.
Künne, 1909. Same case as that reported by Hart.
Datnowski, 1909.
Beitzke, 1910. Same case as that reported by Datnowski.
Moriani, 1910.
Cooke, 1911.
Veszprémi, 1912. Two cases.
Lewis, 1912.
Jonas, 1912.
Beattie and Douglas, 1912-13.

REVIEW OF REPORTED CASES

So far as it is possible to decide, the preceding thirty-eight cases are true cases of periarteritis nodosa. Up to the present time it has been impossible to be sure of the diagnosis without a microscopic examination of the lesion. Consequently, a case such as that reported by Sabin cannot be included in this series, although it may well have been an instance of the affection. In order to make the record complete, several of these questionable cases are given at the end of this article. These have either been reported as periarteritis nodosa or have been considered such by some authors.

The following review is based on a study of the preceding thirty-eight cases. It is at once apparent on reading over the reported cases

that many are lacking in important details. The more detailed report of additional cases will be necessary to bring the clinical picture to completeness. There is sufficient material, however, to bring out many facts of interest and to give a clinical picture which will at least aid in the recognition of future cases.

Historical.—In 1852, Rokitansky, in a paper entitled "The Formation of Aneurysms of the Arteries in General, Except the Aorta, and Most of Its Important Primary Branches, with the Further Exception of the Cerebral Arteries,"[2] gave the first complete gross description of the disease. Thirty-five years later, Eppinger studied and described the histologic changes in the arteries of the mesentery in this case. To Kussmaul and Maier, however, must go the credit for the first complete gross and microscopic description of periarteritis nodosa and its recognition as a distinct disease. They reported a case in 1866 as "A hitherto undescribed peculiar disease of the arteries which is accompanied by Bright's disease and a rapidly progressive general paralysis of the muscles,"[3] and proposed the name by which the disease has since been known.

There is some ground for believing that the disease was grossly recognized as early as 1755 by Michaelis and Matani, and in 1810 Pelletan reported very briefly a case in which he counted sixty-three small aneurysms on the various arteries. These reports are too brief, however, to admit of any conclusions as to the true nature of the process. From the time of Kussmaul and Maier's publication to 1899, but eight additional cases were reported, or an average of about one case in four years. Then, as the disease became better known, the number of cases showed a considerable increase, twenty-eight being reported in the following sixteen years. Five of the cases are from the United States and two from Great Britain, nearly all of the rest being from Germany.

Name of the Disease.—As one might expect, several names have been proposed to supplant Kussmaul and Maier's "periarteritis nodosa." Thus we have "multiple aneurysmen" of Meyer, "das congenitale aneurysma" of Eppinger, "arteritis nodosa proliferans" of Fletcher, "polyarteritis acuta nodosa" of Ferrari, "meso-periarteritis" of Hart and "panarteritis," "arteritis nodosa" or "polyarteritis nodosa" of Beitzke, according to the view of the author as to the nature of the process. While it is possible that some of these names are a little more

2. Aneurysmenbildung sämmtlicher Arterien mit Ausnahme der Aorta, und der meisten ansehnlichen primitiven Aeste derselben, ferner mit Ausnahme der Gehirnarterien.

3. Eine bisher nicht beschriebene eigenthümliche Arterienerkrankung die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellähmung einhergtht.

accurate than the one originally used, the disease has come to be known under the title "periarteritis nodosa" and it seems better to continue this name, at least until the etiology of the disease has been established, rather than to introduce confusion by using one or other of the various substitute titles proposed.

Etiology.—The fact that the etiology of periarteritis nodosa is still quite unknown lends added fascination to the study of the disease and emphasizes the desirability of recognition of cases during life in order that complete bacteriologic studies may be carried out. The history of the etiologic views falls into three main groups.

Syphilitic: Influenced by Virchow's opinion, Kussmaul and Maier were inclined to the view that the disease was a syphilitic affection of the arteries. Chvostek and Weichselbaum, Graf, Schmorl and especially Versé have been the chief exponents of this view, while Müller and Benedict have found it impossible to rule out. Many pages have been written upholding and combating syphilis as the causative factor. It would lead too far afield to go into all the arguments. Suffice it to say that the great majority of observers are opposed to the idea even when they limit themselves to purely histologic studies; the Wassermann reaction has been negative in the few cases in which it has been applied; no one has been able to find spirochetes in the lesions; a syphilitic history fails in most of the cases; in only a very few have any other syphilitic lesions been found at necropsy, and a similar, if not identical, disease occurs in stags as pointed out by Lüpke.

Mechanical: While no one seriously considers this view at the present time it is interesting historically. Meyer, in 1878, believed that the disease was due to high blood-pressure rupturing the media, which was either imperfectly formed or later injured by some toxic substance such as alcohol. Nine years later, Eppinger upheld the view of a congenital debility of the internal elastica with rupture. A variation of the mechanical theory is encountered in Ferrari's idea that the lesion is produced by the central or local action of a toxic substance, such as alcohol, causing a paralysis of the smooth muscle-fibers. All of the facts are against these various ideas and it is scarcely worth while to discuss them.

Infectious or Toxic: All observers, with the exception of those above mentioned, believe that periarteritis nodosa is the result of an acute infection, the arterial lesion being caused by bacteria or their toxins. The nature of the infecting agent is still unknown. Cultural investigations during life have been few and yet it is rather striking that in the four cases in which blood-cultures have been taken the results have been quite negative. In Datnowski's case these were repeated three times and the same was true in our second case, the last

blood taken being plated in ascitic glucose-agar. While in no way conclusive, these results would seem to point against any one of the commoner organisms as the causal factor. Anaerobic cultures have not been taken. In our second case, ascitic glucose-agar shake-cultures were made from the heart's blood post mortem, but also with negative results.

Another striking fact is that the examination of sections of the lesions for bacteria has almost uniformly failed to show any organisms. Graf found a few short rods in his sections, but felt that they could not be considered, as the necropsy was done many hours post mortem. Longcope found a few chain cocci in sections from the kidney, but they were not in relation to the nodules.

In only eight cases have cultures been taken at necropsy. In one instance they were accidentally destroyed, leaving the following seven observations:

Oberndorfer: *Staphylococcus aureus* cultivated from the kidney and spleen.

Datnowski: Streptococcus cultivated from the heart's blood. There was a terminal perforative peritonitis in this case.

Veszprémi: Cultures sterile.

Jonas: Streptococcus obtained from the kidney and an influenza bacillus from the adrenal.

Beattie and Douglas: Streptococcus obtained from the renal hemorrhage.

Lamb, Observation I: Streptococcus from the heart's blood associated with a colon bacillus. Streptococcus from the peritoneal exudate. In this case there was a vegetative endocarditis and terminal peritonitis.

Lamb, Observation II: Streptococcus from the cardiac nodule. *Staphylococcus aureus* from the peritoneum and colon bacillus from the pericardial fluid.

Thus, in all but one case in which cultures have been taken, some organism has been obtained. In five of these it was some variety of streptococcus. In two out of these five there was some complicating condition and in two there was also some other organism isolated. In Longcope's case a few chain cocci were found in the kidney sections, but not in association with the nodules.

In reviewing the etiology, one finds no support for the syphilitic or mechanical theory. The course of the disease, the irregular temperature, the high leukocytosis, the complications and the nature of the lesion all point to some infection. Cultures taken during life have been uniformly negative, but have also been limited in number. The failure to demonstrate any bacteria in the lesions may indicate their early

disappearance as is supposed to be the case in rheumatic lesions. While one is not justified in excluding the occasional finding of such an organism as the *Staphylococcus aureus* or influenza bacillus in post-mortem cultures, the most striking point in the examinations so far made is the occurrence of some variety of streptococcus in five of the seven cases, or in 71 per cent. In another case they were seen in sections. It is for this reason that we have included a full description of the streptococcus isolated in our second case. Animal inoculations with this organism and with material from the nodules have been negative.

At present, one can do no more than call attention to these facts as they have occurred. The final solution rests with the future.

It is contended by some that the disease is not specific, but that it is merely an acute infectious lesion of the arteries due to any one of a number of bacteria.

Heredity.—There is no indication that it plays any part.

Sex.—Of the reported cases, twenty-eight were in males, nine in females and in one the sex is not stated. Thus it would appear that the disease is about three times more frequent in males than in females.

Age.—It is essentially a disease of adolescence and early adult life —71 per cent. of the cases occurring between the ages of 10 and 40. It is not limited to this period, however, the youngest case being in a child 2½ months old, and the oldest occurring at the age of 57. The number of cases in the second decade is slightly larger than in any other. The following table shows the number of cases in each decade:

Years	No. of Cases
1-10	1
10-20	10
20-30	8
30-40	9
40-50	5
50-60	5

Occupation.—This is most varied and it is quite impossible to discover any connection with the affection.

Past History.—In about one-third of the cases this is not given. In only four cases is there a definite history of syphilis. In a few there is a history of symptoms similar to but antedating the symptoms of onset by some months or years. In a few there is a history of some immediately preceding infection. There is thus no apparent relation to any other disease, and one gathers the impression that the previous illnesses are entirely irrelevant. It is, however, interesting to note that some cases do show similar symptoms in their past history either as isolated occurrences or in recurring attacks.

Symptoms.—Onset: In the 32 cases in which this is mentioned, we find a more or less acute invasion in 14 cases and a more gradual onset in 18. The symptoms of onset in order of frequency were as follows: Pains in the muscles or joints, 13; fever, 9; abdominal pain, generally in the epigastrium, 8; edema, 8; weakness, 5; disturbances of sensation, 4; diarrhea, sweating, purpuric eruption, sore throat, 3 each; chilliness, headache, vomiting, anorexia, constipation, cough, 2 each; and scattered symptoms of dizziness, urticaria, pain in the chest, eruptions, hemoptysis, cyanosis, dyspnea, icterus, swelling of the testis and asthma.

General: Mentioned in 32 cases. Weakness, generally progressive, 24; emaciation, 13; sweating, 7; jaundice, 6; sleeplessness, 4; chilliness, 4.

Subcutaneous Nodules: In two cases (Schmorl, Benedict) the diagnosis has been made during life by the examination of excised subcutaneous nodules. Everyone has been impressed with the importance of these nodules and it is generally agreed that they offer the only certain means of diagnosis. And yet they were present only eight times or in about 21 per cent. of the cases. In Kussmaul and Maier's case, small, firm, pea-sized nodules appeared beneath the skin of the abdomen and thorax four days before death. Freund merely states that there were subcutaneous nodules of the face, neck and arms noticed during the patient's eight days in the hospital. Müller (Case 1) found the nodules about one knee at the necropsy.

Schmorl reports very briefly the case of a woman who showed nodules in the abdomen and thigh which on excision were characteristic of the disease. They were apparently cured by antisyphilitic treatment. Mönckeberg noticed a few, small, elastic, painless nodules in the thigh on the day before death. Benedict has described the nodules in more detail. At the end of the fourth week, in his case, a fist-sized tumor appeared in the abdomen and increased somewhat in size. This was finally considered an intramuscular hematoma. At about the same time, four or five hazel-nut-sized, round, painless, easily movable nodules appeared in the skin of the thorax and several pea-sized nodules in one leg and in both forearms. A thoracic nodule showed only clotted blood. Later, eleven pea-sized, round or oval, moderately hard, freely movable, nodules were found under the chin and in the buttocks, arms and legs, three hazel-nut-sized tumors under the left breast and a goose-egg-sized nodule containing pus under the left trochanter. A second small nodule removed from the leg showed changes characteristic of the disease. In Datnowski's case there was slight periosteal thickening of the tibia, and four days before death small elevations of the abdomen appeared. It was suggested that both

these represented subcutaneous nodules, but no examination was made. In the first of the cases here reported, there was a peculiar small firm area in the course of the internal saphenous vein, a raised reddish-brown area 3 by 1 cm., in the right thigh, lying over a superficial vein and the early appearing goose-egg-sized hematoma-like swelling of the ankle. These were not in evidence at the necropsy and so, unfortunately, no examination was made.

Superficial Lymph-Nodes: These were noted as enlarged in only four cases.

Edema: The most striking fact in this connection is that in twenty-one, or 61 per cent., of the thirty-four cases in which histories are given, there was edema at some time during the course of the disease. In only two cases is it definitely stated to have been absent. In the eleven remaining cases in which the histories are more or less completely given it is not mentioned and was presumably absent. The edema was generally of the ankles, less often of the face or body.

Skin Eruptions: In seven cases there was an eruption of hemorrhagic character, petechial, purpuric or ecchymotic and at times this was associated with urticaria. In a few instances, such hemorrhagic spots were the first things noticed at the onset of the disease, at other times they appeared during the course, generally in recurring crops. In one case there was an eruption of uncertain character, considered to be that of either syphilis or scarlet fever. In another there was a desquamation of the whole body typical of scarlet fever, but without any known preceding eruption. In one of our cases there was a rather late diffuse erythema of the face and body.

Pains in the Joints: In fifteen cases, or 39 per cent., there were definite joint-pains, often quite severe, usually involving more than one joint and behaving very much like acute rheumatic fever.

Pains in the Muscles: In eighteen cases, or 47 per cent., there were pains in the muscles, generally of the extremities, usually quite severe and described as "tearing." In three cases these pains were associated with distinct muscular atrophy.

Temperature: In four cases no mention is made of the temperature. In two cases it is definitely stated to have been normal, while in the remaining thirty-two there was fever. Thus 94 per cent. of the cases have shown a definitely febrile course. In some, it is merely stated that there was slight fever, in others it is perhaps a little difficult to separate the fever of the disease from that of the complications. In reviewing the cases in which there is more detailed information, it is apparent that the temperature is generally irregular, at times reaching 104 or even 105 F., and at other times being subnormal. It is apt to be of a remittent type, the remissions being accompanied by marked sweating.

There are often periods of several days when the temperature is quite normal.

Circulatory System: Symptoms and physical signs directly referable to the heart and vessels are rather strikingly absent. Thus in our second case in which the coronary arteries were most extensively involved, there were no signs to direct one's attention to the heart until near the end. In 7 cases there was enlargement of the heart, murmurs in 7, dyspnea in 5, cyanosis in 4, precordial pain in 1 and pericarditis in 1.

Blood-Pressure: There is information on this point in only five cases. In Benedict's case it was 140, in Datnowski's 215, in Kunne's 77 to 100, in the first of our cases 130 to 165, and in the second 104.

Pulse: In five cases the pulse was not mentioned, and in one it was quite slow. In the remaining thirty-two, or 97 per cent., it was increased in frequency, and in sixteen, or in one-half of the cases in which it was increased, it was more rapid than one would expect from the height of the temperature.

Blood: Anemia is a striking feature of the disease. It is stated definitely as being present in 55 per cent. of the cases and is generally described as being most conspicuous. In only five cases is there any record of a red cell-count or hemoglobin estimation. In these counts the red cells varied from 3,500,000 to 2,800,000, and the hemoglobin from 75 per cent. to 42 per cent. Leukocytosis: Observations have been made on the white cells in only twelve cases. Leukocytosis failed in only one case, that of Dickson, in which there was an associated tuberculosis of the lymph-glands and lungs. In two cases it is simply stated that there was a leukocytosis. In the nine cases in which the figures are given, the count varied from 9,000 to 66,000. The low count was in a case in which other, later counts were as high as 24,000. The count is generally high, between 16,000 and 30,000. In only four cases were differential counts made. Of special interest in this connection is the fact, first mentioned by Datnowski, that the eosinophils are increased. In his case they were 10 per cent. In Lewis' case they were from 22 to 30 per cent., and in our first case from 32 to 58 per cent. In all of these cases the usual causes for eosinophilia were excluded. In our second case they were not increased. It is most important to have more observations on this most interesting point, as it may furnish a most valuable means of diagnosis. It is rather significant that the cellular infiltration of the arterial lesions is generally rich in eosinophils. The polymorphonuclear leukocytes are increased in number. Blood-Cultures: Taken in only four cases and sterile in all. In Datnowski's case they were repeated three times. In our second case, three different cultures were taken and one of these was plated in

ascitic glucose-agar. Wassermann Reaction: Taken in only three cases and negative in two. In our second case one test was positive and one was negative. The first was taken when the temperature was high. We have become very suspicious of the accuracy of positive reactions in the presence of high fever. The second test we believe more reliable, as the temperature was low. In one case a negative reaction was obtained with cadaver blood.

Respiratory System: The respiratory symptoms bear very little relation to the disease with the possible exception of the asthma, which is at times cardiac, and the blood in the sputum. In one of our cases asthma was a most striking feature from the outset, and in one other of the reported cases it was severe. In three cases there has been blood in the sputum. The other symptoms have been the following: cough in 8; pulmonary edema, pneumonia, pain in the chest, 2 each; pleural effusion, emphysema and hemoptysis, 1 each.

Gastro-Intestinal System: A large number of the cases show some symptoms referable to the gastro-intestinal system. In order of frequency they have been the following: Vomiting, 17; anorexia, 9; constipation, 7; diarrhea, alternating with constipation, 5; diarrhea, 4; blood in the stools, 1; *Ascaris lumbricoides,* 1. In the only 3 cases in which gastric analyses were made the free hydrochloric acid was either reduced or entirely absent.

Abdomen: The abdominal symptoms are most important. It is this aspect of the disease which is of interest to the surgeons, for at times the symptoms have been severe enough to persuade one that an acute surgical condition existed. The second case here reported is a striking illustration of this. In seventeen cases, or 50 per cent. of those in which the facts are given, there was abdominal pain. In one-half of these it was located in the epigastrium or upper part of the abdomen. In the rest it was generally diffuse over the whole abdomen. In three cases it was in the region of the kidney. In eight cases the abdominal pain was one of the marked symptoms of onset. The character of the pain is fairly constant. In most cases it comes on in recurring attacks and is severe, colicky or cramp-like. At times it is severe and constant for several days at a time. In a few cases it radiates to the back. It is generally associated with abdominal tenderness and distention. In seven cases the liver was palpable and in four cases the spleen could be felt.

Urogenital System: The lesion affects the small arteries of the kidney in nearly every case. Therefore, the symptoms referable to the kidney are most important. In ten cases no mention is made of the urine. In five cases it is definitely stated that albumin was not present. Often, however, there was only one examination of the urine and the

condition of the kidneys at necropsy suggested that, in some of the cases at least, albumin would have been found had repeated examinations been made. In twenty-three cases, or eighty-two per cent. of those in which the condition of the urine is given, albumin was present, generally in moderate amount, but varying from a faint trace to as high as 6 per cent. In the cases in which repeated examinations were made, the albumin was not always present but showed exacerbations and remissions. It was sometimes present at the onset, clearing up as the patient improved and reappearing later. In other cases it was not present until near the end. In fourteen cases, casts were present. These were generally of the hyaline and granular variety, but occasionally they were epithelial. The amount of the urine was reduced in nine cases and in a like number blood was present. In one case there was a faint trace of sugar found. In one of our cases the phenolphthalein output for two hours was too small to estimate on two occasions. The necropsy showed a very severe glomerulonephritis. In the other case the test was not done. In two cases there was infarction of the vessels of the testis.

Nervous System: The following symptoms were noted: Headache, 6; disturbances of sensation, 6; palsies, 4; convulsions, 4; visual disturbances, 3. In one case in which lumbar puncture was done the spinal fluid was sterile and in one of our cases the urea content was 0.323 per cent. In one of the cases showing visual disturbances a lesion was found in one of the retinal arteries. In the other two cases the retina was not examined post mortem.

Pathology.—The lesions of periarteritis nodosa consist in the peculiar arterial nodules and aneurysms and the changes in the various organs dependent on an insufficient or completely obliterated blood-supply. The larger arteries are not affected, the changes being limited to those of the size of the coronary and smaller. The veins and capillaries have never been found affected. The distribution of the lesions is practically that of the small and medium-sized arteries, that is, any arterial area may be involved. There are certain peculiarities, however, in that the arteries of the brain and spinal cord and the pulmonics are very rarely affected. Changes of the former have been found only four times and of the latter only twice. In some of the cases the central nervous system has not been examined. While the pulmonary arteries almost regularly escape, it is not at all uncommon to find the lesion in the bronchial arteries. The lesion has been found in practically every region of the body, including the retina, arteries to the nerves, the muscles, subcutaneous tissue, spermatic cord, diaphragm, etc. In nearly every case there are lesions in the heart, gastro-intestinal tract and kidneys. In 1905, Mönckeberg gave a table showing the site of the

lesions in all the cases published up to that time. It does not seem necessary to enlarge on this table at present, especially as in many of the cases complete examinations were not made and all of the vascular areas were not investigated. Consequently, a table gives a rather erroneous impression.

Two important facts should be borne in mind. In the first place, in no less than five cases, no changes were noted in the arteries at the necropsy and the disease was not suspected until demonstrated in the sections. It is therefore possible that some cases may be overlooked both clinically and pathologically. That this is not often the case and also that the disease is in reality quite rare is shown by the fact that in a recent review of the sections of all cases of nephritis occurring at the Presbyterian Hospital during the past six years, no case of periarteritis nodosa was found, although the disease was carefully looked for.

In the second place, in five cases, the disease was limited to a relatively small vascular area or to one organ. Some time ago this was used as an argument against such a case being truly periarteritis nodosa, but it is now well recognized that such limitation may occur.

The gross appearance varies somewhat. In most cases there are both solid nodules and aneurysms. These occur with especial frequency at the points of branching, the size of the nodules having no relation to the size of the artery. The nodules are whitish to yellowish-white, moderately firm, later quite firm, and vary in size from those just visible and resembling miliary tubercles to those the size of a pea or slightly larger. They are generally situated at one side of the artery, but at times the whole wall of the artery seems thickened. As indicated above, many of the lesions are quite invisible to the naked eye. On section, they appear homogeneous, yellowish-white, and often show an associated occlusion of the artery. The larger nodules are generally aneurysmal dilatations, are generally softer in consistency and vary from a yellowish-white to a grayish-red or dark-red. These are almost regularly filled with thrombus and may slightly exceed a hazel-nut in size. The aneurysms and nodules may occur together on the same artery and often occur in thickly set bunches or are strung along the branch, giving it a corded or nodular feel. There may be petechial hemorrhages over the lesions and hemorrhagic extravasations into the surrounding tissues. The aneurysms may rupture resulting in hematomata. Quite regularly one finds infarcted areas in some of the organs from occlusion of the arteries at the site of the lesion. The portions of the arteries in which there are no nodules show no changes. The lesion may be quite early in one region and more advanced in another.

Perforation of the intestine may occur through an ulcer caused by infarction.

Microscopic Findings.— One of the chief points of controversy has been the question as to which coat of the artery is the seat of the primary change. The majority of observers believe that the disease begins in the adventitia, a fair proportion are equally insistent that the media represents the primary change. While there have been a few exponents of a primary intimal inflammation, all recent observers are agreed that the process does not begin there. It is believed that the injurious agent probably enters by way of the vasa vasorum or possibly the perivascular lymphatics, and affects one or other of the two outer coats. The reason that there has been so much difference of opinion concerning the relative degree of involvement of the different coats is that the lesion varies considerably in various portions of the same nodule and in the early and late stages of the disease. It is only by the study of serial sections and a review of the various histologic descriptions in the different cases that one can obtain a proper idea of the whole picture. Summing up these changes, we find the picture in the various coats as follows:

Intima: Is frequently not involved. No one has found changes here without simultaneous changes in the other coats. It is on this basis especially that the intima has been ruled out as the site of primary change. As a rule, with the changes in the other coats there is some degree of involvement of the intima. At times there is simply a proliferation of the endothelial cells, at times this is sufficiently marked to cause narrowing of the lumen. The cells then often appear large and polygonal in shape with clear, spindle-shaped or irregular nuclei; the tissue appears vacuolated; there may be some edema and fibrin with an occasional polymorphonuclear leukocyte and wandering cell and some nuclear fragments. A little later fibroblasts appear and with the formation of thrombi these cells grow into and organize the thrombus mass, at times leading to complete fibrous occlusion of the vessel. The changes may affect only a portion of the circumference or the whole intima. The cellular exudate of the media and adventitia may break through the internal elastica and invade the intima. The intimal thickening may extend for some distance beyond the nodule.

Internal Elastica: Generally at the site of the lesion it is wanting. In some cases it is quite sharply broken, the ends tending to curl up; more often it fades away into the exudate of the other coats. Often one can find it quite intact on the other side of the lumen. It may show definite small breaks in continuity in this region. At times it appears distinctly swollen at the site of the nodule.

Homogeneous Hyaline Ring: This is a very characteristic peculiarity of the lesion and consists of a zone of varying width, of more or less homogeneous appearance which takes the eosin stain quite intensely. It is generally situated just external to the internal elastica which is often preserved. In some cases it has been described as lying internal to the elastica and, as mentioned in one of our cases, it may lie both external and internal to it. In the more advanced lesion the band or ring is hyaline and structureless in appearance; but it seems quite possible to trace its formation from the degenerated media. Certainly, in the early stages the inner portion of the media can be seen to be more or less degenerated, its elements fusing together and taking the eosin stain more intensely. At a little later stage one sees the hyaline ring in this location quite distinctly, but it is quite possible to make out the degenerating smooth muscle cells within it.

Media: This coat is almost regularly involved. Mönckeberg even goes so far as to say that when no changes are demonstrable they really exist and that our present means of examination are not delicate enough to make them out. The early changes consist of swelling of the muscle-cells with a loss of their nuclei. One then finds a beginning inflammatory exudate of polymorphonuclear leukocytes, wandering cells and eosinophils, increasing to an extensive similar exudate with some fibrin, edema, nuclear fragments and vacuolization with increasing degeneration of the media, the formation of the hyaline band and later the appearance of fibroblasts and the gradual replacement of the exudate by fibrous tissue. At times, as in the aneurysm formation, the media is greatly thinned. The external elastica shows changes comparable to the internal.

Adventitia: An increasing number of observers have found changes in this coat without the occurrence of recognizable lesions elsewhere in the arterial wall. The earliest change consists of a small periarterial or adventitial collection of mononuclear wandering cells, polymorphonuclear leukocytes and eosinophils. I have been rather impressed with the occurrence of the eosinophils. It has been recognized for a considerable time that they are numerous in the lesions. In our sections, they seem attracted to the region of the arteries even when these show no change. The earliest lesion seen in our sections was in a small arteriole of the stomach-wall. This showed a small unilateral periarterial collection of cells almost all of which were eosinophils. With the older lesions the infiltration of cells increases until the adventitia becomes several times its normal width and merges imperceptibly with the periarterial infiltration which may extend for some distance into the surrounding tissue. The adventitia shows a beginning degeneration and then goes on to the formation of granulation tissue and finally

fibrous tissue. At times one finds the perivascular lymphatics filled with an exudate similar to that described, never, however, to the same extent as seen in such streptococcus infections as erysipelas. The lesions do not go on to suppuration, and in this respect resemble the lesions in the organs in *Streptococcus viridans* endocarditis.

The lesion bears no relation to the supra-arterial epicardial fibroid nodules described among others by Knox. On gross examination these nodules are somewhat suggestive of periarteritis nodosa, but their histologic structure is quite different.

Brief mention has been made above to the occurrence of a similar disease in stags. Lüpke first described this condition in 1906. There has been some divergence of opinion as to the exact relationship between the two conditions. The descriptions of the lesion are rather brief and as we have had no opportunity of seeing sections taken in the cases we feel in no position to judge of their similarity. The consensus of opinion seems to be that the two conditions are very similar if not identical.

Duration.—The most acute case resulted in death in ten days. One case was followed for two years and ten months and was, as far as I have been able to discover, lost sight of at the end of that time. In general, the course is relatively rapid, 71 per cent. of the cases resulting fatally inside of three months. The duration is not always accurately given and in the following table I have considered only the period of acute symptoms.

Duration	No. of Cases
Not given	3
Less than 1 month	5
1-2 months	10
2-3 months	10
3-4 months	5
6-7 months	1
7-8 months	1
8-9 months	1
Over 1 year	2

Prognosis.—This is bad. Only one case in the series did not result fatally. This patient was followed for nearly three years and was alive at the end of that time. One patient in whom the diagnosis was made during life by the examination of an excised nodule was apparently cured of the disease, but died two years later of portal thrombosis The necropsy showed no evidence of periarteritis nodosa except some small fibroid foci in close relation to the arteries. These were considered healed nodules. Other cases have shown similar but less marked tendency to healing in some regions, but always with progression of the process elsewhere.

Diagnosis.—One need but glance at the following list of diagnoses which have been made in the foregoing cases to obtain some idea of the puzzling nature of the disease: nephritis 7, meningitis 3, general miliary tuberculosis 2, general peritonitis 2, sepsis 2, dysentery, trichiniasis, morbus maculosus Werhlohii, encephalitis, typhoid, polymyositis, polyneuritis, dermatomyositis, cerebral hemorrhage, appendicitis and Schönlein's purpura rheumatica. One thing which one notices about these diagnoses, in reading over the cases, is that they were not considered quite typical and that they did not fully explain the picture.

While some few observers are inclined to the belief that the disease is not a distinct entity, but that it is merely an unusual incident in the course of some acute infectious disease, the majority of those who have studied it clinically and pathologically have come to the conclusion that periarteritis nodosa is a disease *sui generis*. Until the etiology is cleared up this point cannot be definitely determined; but the facts seem to point to the latter point of view.

The difficulty in arriving at a symptom-complex on which a diagnosis may be made lies in the wide-spread distribution of the lesions throughout the arterial system, with the corresponding symptoms in one or more regions or organs. Add to this the tendency of the disease to spread to new vascular areas and the occurrence of numerous overshadowing complications and one can appreciate the difficulties in the way of reaching a correct diagnosis.

Is it, then, possible to construct a symptom-complex of periarteritis nodosa sufficiently definite to make a diagnosis possible? Such a diagnosis has been correctly made only twice during life and then by the excision and examination of the subcutaneous nodules. Barring the presence of these nodules, an answer to the foregoing question must be in the negative. I believe, however, that a consideration of certain symptoms and groups of symptoms should make it possible to suspect the disease and should lead to more careful examinations for subcutaneous nodules. When the disease is once suspected every possible examination should be made to aid in clearing up the etiology. Since the occurrence of the above cases in the hospital, there have been at least two cases in which periarteritis nodosa has been suspected. In both of these there was a purpuric eruption and in both some of the spots were excised for microscopic examination. Neither case turned out to be an instance of this affection.

In practically all of the cases there is fever of a more or less irregular type, rapid pulse, often disproportionately so, anemia and progressive weakness and a striking leukocytosis with a high percentage of polymorphonuclears and in some, at any rate, a distinct eosinophilia. It is most important to have this eosinophilia confirmed by future

observations, for if it be at all constant it will furnish a most valuable means of diagnosis. The above facts stand out clearly and represent a pretty definite background against which one must form the clinical picture. Of almost equal importance, even if not of quite as common occurrence, is the presence of edema and the symptoms of nephritis. While one would, of course, hesitate in making any diagnosis on these symptoms one should be equally reluctant in suspecting periarteritis nodosa without them. If one finds, in addition, some of the following less common but quite frequently present symptoms, it seems justifiable to suspect the disease: pains of tearing character in the extremities; pain and swelling of various joints; attacks of acute abdominal pain, colicky in character and generally located in the upper abdomen; vomiting; hemorrhagic eruptions; cyanosis; asthma. If subcutaneous nodules appear, the diagnosis can be easily settled by excision and histologic examination. In addition it should be remembered that the course of the disease is relatively short and that there may be distinct periods of remission of symptoms and improvement.

OTHER DOUBTFUL CASES

In addition to the list of cases considered in the preceding review, there are a few which have many points in common with periarteritis nodosa and which have been included in reviews of the disease by some authors. Some of them are possibly and even probably instances of this affection, but the failure of histologic examinations makes a decision impossible. The authors of these cases with the dates of publication are the following: Gee, 1871; Thompson, 1877; Sabin, 1901. In addition, there are the cases of Matani, Michaelis and Pelletan already referred to. The cases of Geipel and Eichorst are apparently not cases of this affection.

146 East Seventy-Fourth Street.

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Veszprémi and Jancsó: Beitrage, Ibid., 1903, xxxiv, 1.
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Von Kahlden: Ibid., 1894, xv, 581.
Zimmermann: Arch. d. Heilk. (Wagner), 1874, xv, 167.

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Zur biologischen Bedeutung der ungesättigten Fettsäuren.

Von **James W. Jobling** und **William F. Petersen.**

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Da es das leitende Ziel aller biologischen Forscher ist, besonders auf medizinischem Gebiete, durch tieferes Eindringen in das Wesen der chemischen Zusammensetzung biologischer Reaktionskörper etwa ihren Reaktionsunterschied auf einfache physio-chemische Prozesse abzuleiten, möchten wir es nicht unterlassen, eine kurze Abfassung verschiedener Studien, welche in dieser Beziehung von Interesse sein könnten, vorzulegen.

Bekannterweise haben die Fettkörper im allgemeinen eine einfache chemische Zusammensetzung; vielleicht gerade deswegen hat man ihnen weniger Beachtung gewidmet, da man ja gewöhnlich komplizierte Prozesse mit komplizierten Substanzen einzuschalten sucht. Unter den verschiedenen Fettsäuren besteht aber an und für sich ein Unterschied, welcher von prinzipielle Bedeutung ist; nämlich das Vorkommen der ungesättigten Kohlenstoffatome in einer großen Anzahl solcher Säuren. Diese chemische Gruppierung ist bekanntlich sehr aktiv. Diesem Unterschiede wird aber in der medizinischen Literatur wenig oder keine Bedeutung beigelegt. Außerdem wird diesen Fettsäuren durch ihre physikalischen Eigenschaften eine besondere Reaktionsfähigkeit zuteil, indem sie sich leicht verseifen und dadurch wasserlöslich werden und als Seife, wie auch als Fettsäure, unter sich löslich sind; auch ändern sie durch Reaktionsunterschiede, wie auch durch Einfluß von Wärme und Kälte, ihren Dispersitätsgrad und werden inaktiv oder gelegentlich auch stärker reaktiv. Dazu kommt noch ihr intimes Verhältnis zu den Eiweißkörpern, welches in vielen Fällen erst durch tief eingreifende chemische Mittel gesondert werden kann. Auch als Ester, und in Verbindung mit Lecithin,

Cholesterol und anderen dergleichen Lipoidkörpern treten Fettsäuren als aktive Bestandteile immer wieder zutage.

Unsere Studien mit den Fettsäuren nahmen ihren Ursprung von einer Untersuchung über den Mechanismus der tuberkulösen Verkäsung. Da es in diesem Zustande gewöhnlich nicht zu einer Auflösung der verkästen Gewebe kommt, muß man annehmen, daß die Fermente der Selbstverdauung entweder abwesend, oder durch irgend welche Hemmungskörper paralysiert werden. In Anbetracht des großen Lipoidgehalts der Tuberkelbacillen lag es auf der Hand, den Einfluß dieser Körper auf die tryptische und leukoproteolytische Verdauung zu prüfen.

Methode.

Wir erkannten sehr bald, daß die Gross-Fuldsche Methode für genaue Resultate absolut ungeeignet ist, da eine exakte Ablesung eines unverdauten Kaseinrestes auf der Willkür des Beobachters beruht, ganz besonders wo noch Lipoide anwesend sind. Es ist uns oft vorgekommen, daß wir nach der Gross-Fuldschen Methode eine vollständige Hemmung vermuteten, aber es stellte sich bei der darauffolgenden Stickstoffbestimmung heraus, daß 30—60 Proz. verdaut waren. Um wirklich einwandfreie Resultate zu erhalten, haben wir in jedem Experimente nach sorgfältiger Ausfällung und Filtration Stickstoffbestimmungen an dem verdauten Kasein gemacht. Zu diesem Zwecke haben wir von der ausgezeichneten mikrochemischen Methode Folins (1) Gebrauch gemacht, eine Methode, welche durch ihren geringen Anspruch an Material, durch Zeitersparnis und durch Zuverlässigkeit sich ganz besonders zu biologischer und auch serologischer Arbeit eignet. Unsere Resultate zeigen also immer den ganauen Prozentsatz der Verdauung; eine subjektive Beeinflussung bei der Ablesung ist völlig ausgeschlossen.

Fermenthemmungskörper aus Tuberkelbacillen.

Wir extrahierten die Fettkörper der Tuberkelbacillen durch Aether, Petrol und Alkohol im Soxhletapparate. Die verschiedenen Fraktionen — Fettsäuren, höhere Alkohole etc. — wurden als feine Emulsion in Salzlösung gebracht und nun wurde Trypsin zugesetzt. Nach halbstündigem Stehen im Brutschranke wurde Kasein zugefügt und nach vollendeter Verdauungzeit wurde ausgefällt. Wir fanden keine Inhibition durch vorhandene Lipoide. Nun untersuchten wir, ob durch Verseifung eine Inhibition zu erzielen wäre. Dies wäre möglich, indem die Fettsäuren mit dem Ferment in weit intimere Beziehung

treten könnten. Es stellte sich sofort heraus, daß solche verseifte Fettsäuren der Tuberkelbacillen das tryptische wie auch das leukoproteolytische Ferment bedeutend hemmten, so z. B. wurde durch 1 ccm einer 1-proz. Seifenlösung die Verdauung vollständig aufgehoben. Trennten wir nun die gesättigten von den ungesättigten Fettsäuren, so fanden wir, daß die antitryptische Eigenschaft den ungesättigten Fettsäuren zukam; dagegen verloren sie ihre Aktivität durch Jodabsättigung (2).

Seifen als Hemmungskörper.

Wir untersuchten nun verschiedene Seifen aus Hanf und Lebertran und aus Oliven-, Croton-, Leinsamen- und Rizinusöl, auf ihr antitryptisches Vermögen und fanden, daß dieselbe im direkten Verhältnis zu der Jodzahl der respektiven Fettsäuren stand. Die einzige Ausnahme machte die Seife aus Rizinusöl, welche weniger hemmte als man ihrer Jodzahl gemäß erwarten konnte. Dieses Resultat läßt sich wohl durch den Unterschied in der chemischen Zusammenstellung der Fettsäuren des Rizinusöles erklären, da in diesem das ungesättigte Kohlenstoffatom sich nicht wie in den anderen Fettsäuren in der direkten Kohlenstoffkette befindet und auch weit weniger reaktiv sein soll. Wir fanden, daß diese Seifen durch Jodabsättigung wie auch durch Absättigung mit verschiedenen anderen Methoden ihre Hemmungskraft einbüßten; so z. B. beobachteten wir, daß eine Seife aus Leinsamenöl in einem Monat fast die Hälfte ihrer antitryptischen Kraft, bei einfachem Stehen bei Zimmertemperatur, verlor. Indem es uns nie gelungen ist, das Trypsin nach Behandlungen mit den ungesättigten Seifen wieder zu reaktivieren, muß man wohl eine Zerstörung des Trypsins annehmen.

Wir untersuchten auch verschiedene andere Lipoide, z. B. Cuorin, Cholestero und verschiedene Lecithinpräparate. Doch konstatierten wir eine Hemmung nur an solchen Lecithinpräparaten, in welchen man von ihrer Jodzahl die Anwesenheit von ungesättigten Fettsäuren annehmen konnte. Die Hemmung erreichte aber nie denselben Grad wie bei einer Seifenlösung mit derselben Jodzahl.

Der Unterschied zwischen den gesättigten und ungesättigten Seifen als Hemmungskörper ist im folgenden Experiment

sehr deutlich zu sehen. Die Hydroxy-Palmitinseife, welche wir hier brauchten, wurde aus der Oelseife hergestellt (3).

No.	Kasein ccm	Trypsin ccm	Na-Oleate ccm	Na-Hydroxy Palmitate ccm	Stickstoff, verdaut mg	Proz.
1	2,0	0,2	0	0	1,66	100
2	2,0	0	0	0	0	0
3	2,0	0,2	2,0	0	0,12	7
4	2,0	0,2	1,0	0	0,12	7
5	2,0	0,2	0,75	0	0,18	10
6	2,0	0,2	0,50	0	0,2	12
7	2,0	0,2	0,25	0	0,26	15
8	2,0	0,2	0	2,0	1,52	91
9	2,0	0,2	0	1,0	1,63	100
10	2,0	0,2	0	0,75	1,66	100
11	2,0	0,2	0	0,50	1,60	100
12	2,0	0,2	0	0,25	1,66	100

Kaseinlösung 1 Proz., Na-Carbonat 1 Proz., Trypsinlösung 0,2 Proz., Seifenlösung 1 Proz.

Die tuberkulose Verkäsung.

Nachdem wir gefunden hatten, daß die verschiedenen ungesättigten Seifen, sowie auch die Seifen aus Tuberkelbacillen (Jodzahl 24) antitryptisch wirksam waren, untersuchten wir die Verhältnisse der Fermente und Antifermente in verkästem Drüsen und Lungenmaterial. In der Verkäsung handelt es sich offenbar um eine Hemmung des autolytischen Prozesses und es lag auf der Hand, die Anwesenheit der ungesättigten Fettsäuren als Hemmungskörper zu erforschen. In echtem verkästen Drüsenmaterial fanden wir nie eine Selbstverdauung, wie auch schon Schmoll (4) gezeigt hat. Solches Material wird von Trypsin nur verdaut, wenn letzteres in großem Ueberschuß vorhanden ist. Wir extrahierten solches Material auf längere Zeit im Soxhletapparat mit Aether und Chloroform. Der gesamte Aether-Chloroformrückstand wurde verseift, und es stellte sich heraus, daß diese Seifen ganz bedeutend antitryptisch wirkten. Dagegen fanden wir, daß sich nun das extrahierte Drüsenmaterial leichter durch Trypsin verdauen ließ als das Originalmaterial. Gelegentlich fanden wir auch, daß das Drüsenmaterial seine antitryptische Eigenschaft durch einfaches Auslaugen mit Salzlösung zum Teil verlor und daß man durch die Verseifung der so gewonnenen Flüssigkeit be-

deutende Hemmung erzielte. Daraus muß man wohl die Anwesenheit der ungesättigten Fettsäuren als Seifen annehmen, doch wird wohl der größte Teil der antitryptischen Eigenschaft auf den Lipoidverbindungen der ungesättigten Fettsäuren beruhen.

Durch Jodvorbehandlung des verkästen Materials wird die Verdauung durch Trypsin in 24 Stunden fast verdoppelt. Diese Beschleunigung läßt sich wohl in der teilweisen Absättigung der Fettsäuren und ihrer darauf erfolgenden Inaktivierung erklären. Die therapeutische Beeinflussung der Tuberkulose durch Jod hat also eine leicht verständliche Basis, indem durch Herabsetzung der Antifermente die verkästen Gewebe dem fermentiven Angriffe freigestellt werden. Klinisch findet diese Verdauung ihren Ausdruck in dem Erscheinen von Tuberkelbacillen (meist intrazellulär) im Sputum und einer erhöhten „Eiweißzerfalls"-Intoxikation (besonders bei avancierten Fällen). Anbetracht der bedeutenden antitryptischen Wirksamkeit der Oelseife scheint die Immunisierung mit in Oelseife gelösten Tuberkelbacillen sehr rationell, indem dadurch die toxischen Erscheinungen des Bacilleneiweißes vermindert werden ohne Beeinträchtigung der antigenen Kraft (Zeuner, 5, 6).

Serum Antitrypsin.

Bekannterweise wird die antitryptische Eigenschaft des Serums durch die Einwirkung des Chloroforms aufgehoben, wie auch einer von uns (Jobling) schon vor einigen Jahren feststellte und verschiedentlich in der Literatur erwähnt worden ist. Durch die einfache Zerstörung der Extraktion des Antitrypsins durch die Fettlösungsmittel wurde allerdings die Aufmerksamkeit der verschiedenen Forscher auf den Lipoidbestandteil des Serums gelenkt, doch da man das Antitrypsin nie aus den Lösungsmitteln erhielt, ließ man die Idee des lipoiden Charakters des Antitrypsins fallen und schloß sich der Idee Rosenthals an oder erklärte sich das Antitrypsin als echten Antikörper.

Da sich das Antitrypsin im Serum in kolloidaler Lösung befindet, kann man wohl schwerlich dieselbe Hemmungskraft durch eine grobe Aufschwemmung der extrahierten Serum-

lipoide erlangen, indem die Lipoidemulsion mit dem Trypsin in keine intimere Beziehung treten kann. Dieser Dispersitätsunterschied ist von wichtigster Bedeutung, ist aber nicht nur hier, sondern auch in manchen anderen Fällen von biologischen Forschern nicht berücksichtigt worden. In diesem Falle kann man durch einfache Verseifung dieser Fettkörper den Originaldispersitätsgrad wieder erlangen und durch diese Behandlung ist es uns ermöglicht worden, die vollständige Hemmungskraft des Serums in dem Chloroform-, bzw. Aetherrückstand nachzuweisen.

Die im Chloroform gewonnenen Lipoide und freien Fettsäuren enthalten alle ungesättigte Fettsäuren und haben eine Jodzahl von etwa 50. Durch Jodabsättigung, sowie auch durch Einwirkung einer starken Wasserstoffperoxidlösung schwindet die antitryptische Eigenschaft des Serums. Auch Jodkalium und Jodnatrium heben die antitryptische Wirkung auf, wenn man dieselben ungefähr in dem Verhältnisse von 10 Proz. im Serum löst und auf 24 Stunden in den Brutschrank stellt; doch mag es sich hier um eine einfache Salzhemmung der Trypsin-Antitrypsinverbindung handeln. Die Möglichkeit ist immerhin vorhanden, daß durch Freiwerden von Jod es zu einer tatsächlichen Absättigung der ungesättigten Fettsäuren kommt. Auch scheint die Beeinflussung durch die Jodsalze an frischem Serum mehr ausgeprägt als an älteren Sera.

Eine Beobachtung von v. Dungern und Hirschfeld (7), welche vorliegt, ist von Interesse. Sie beobachteten, daß jodiertes Serum nicht nur autolysierte, sondern auch von Trypsin verdaut wurde. Indem sie die Jodwirkung direkt auf das Eiweiß vermuteten, konnten sie sich mit darauf gerichtetem Experiment keine Erklärung schaffen. Bezieht man aber die Wirkung auf das Antiferment, wie es tatsächlich der Fall ist, so klären sich die Verhältnisse vollständig (8).

Serotoxin.

Es stellte sich nun die Frage, ob das antitrypsinfreie Serum auch im Körper abgebaut werden könne und etwa im homologen Tiere toxische Erscheinungen hervorrufen würde. Wir untersuchten zuerst Meerschweinchenserum, welches vor

dem Experiment durch Ansäuren oder Erhitzen (70° C 30 Min.) inaktiviert wurde; es traten aber bei der intravenösen Einspritzung keine oder nur minimale toxische Erscheinungen hervor. Doch da diese Inaktivation des Antitrypsins wohl auf einem Dispersionsunterschiede beruht und dabei die Lipoide nicht aus dem Serum entfernt werden, wäre es wohl möglich, daß dieselben bei der Injektion noch schützen könnten. Nimmt man dagegen angesäuertes Serum und filtriert etliche Male durch einen Berkefeldfilter, so wirkt das Serum höchst toxisch, wie auch schon Bordet und Doerr gelegentlich fanden. Es genügen schon 3—4 ccm, um damit anaphylaktische Symptome akut zu töten. Gelegentlich fanden wir, daß das wiederholte Filtrieren des angesäuerten Serums durch hartes Filterpapier genügte.

Auch das jodierte Serum (v. Dungern und Hirschfeld) verursacht typischen anaphylaktischen Shock. Behandelt man dagegen Meerschweinchen mit Jodtinktur (auf die rasierte Bauchhaut gestrichen) so gehen die Tiere nach 10—24 Stunden an Jodvergiftung zugrunde. Das Antitrypsin des Serums ist bedeutend vermindert. Das Serum solcher Tiere, welches kein freies Jod enthält, zeigt sich bei der Reinjektion als höchst toxisch, indem sämtliche Tiere schon an einer Dosis von 3 ccm binnen 8 Stunden zugrunde gingen. Die Sektion macht es wahrscheinlich, daß der Tod nicht durch Jodvergiftung, sondern durch eine Eiweißvergiftung verursacht wurde.

Am auffallendsten sind aber die Resultate, welche man durch einfache Chloroform- oder Aetherextraktion erzielen kann. Solche Sera sind für homologe Tiere (Meerschweinchen, Kaninchen) sehr giftig; ein Präparat, welches 24 Stunden unter Chloroform stand, erreichte eine M. L. D. von 0,3 ccm für ein Meerschweinchen von Mittelgröße. Bei der Herstellung solcher toxischer Sera empfiehlt es sich, das Serum mit etwa dem doppelten Quantum Chloroform kräftig zu schütteln, bis eine feine Emulsion entsteht und diese auf 24 Stunden stehen zu lassen. Am nächsten Tage wird scharf zentrifugiert, das milchartige Serum so lange durch Papier filtriert, bis es vollständig klar und chloroformfrei ist. Die Giftigkeit solcher Sera steigt in den ersten Stunden nach Chloroformzusatz sehr

schnell, erreicht seinen Höhepunkt nach etwa 24 Stunden und wird dann bald atoxisch. Das Antitrypsin ist gewöhnlich nach 1 Stunde in das Chloroform übergegangen und es folgt deshalb eine progressive Autolyse des Serums, welche sich leicht feststellen läßt. Bei Zimmertemperatur nimmt der ganze Prozeß längere Zeit in Anspruch, so daß die höchste Toxizität etwa nach 5—6 Tagen erreicht wird, um darauf in den nächsten Tagen bald zu verschwinden.

Indem bei den frisch präparierten Seren eine sofortige Herzthrombose den Tod herbeiführt und in dieser Beziehung unsere Präparate den Cytotoxinen ähnlich sind, haben wir den so gewonnenen Seren den Namen Serotoxin beigelegt. Es handelt sich aber nicht nur um eine Blutgerinnung als Todesursache. Dafür sprechen folgende Tatsachen. Bei der Injektion einer M. L. D. eines solchen bei Zimmertemperatur präparierten Serums findet man gewöhnlich keine Gerinnung, sondern das typische Bild eines anaphylaktischen Shocks. Gleichzeitige Einspritzung von Na. citricum, welches die Gerinnung verhindert, verhindert den Tod nicht, da derselbe durch akute Lungenblähung verursacht wird. Auch hirudinizierte Tiere sind nicht gegen das Seratoxin geschützt, da auch hier der Tod mit akuter Lungenblähung stattfindet; dagegen konnten wir den Tod durch die Beimischung zu dem Serotoxin der extrahierten Lipoide (verseift) immer verhüten.

Das Serotoxin scheint thermolabil zu sein, da Erhitzung bei 70° C für 30 Minuten die Giftigkeit herabsetzt. Man kann aber aus dem Serotoxin stets toxische Spaltprodukte, welche sich fast so giftig als das ursprüngliche Serum erweisen und unter typischen anaphylaktischen Symptomen töten, isolieren. Diese Spaltprodukte (primäre Proteosen) erwiesen sich als koktostabil.

Die Temperaturkurve der injizierten Tiere scheint sich genau wie bei der Anaphylaxie zu verhalten. Ueberlebt das Tier die Injektion, so steigt der Antitrypsingehalt des Serums ganz bedeutend, und solche Tiere sind bei der Reinjektion, wenn auch nicht vollständig, so doch zum Teile geschützt. Auch die Narkose scheint zu schützen, doch nicht in dem-

selben Grade wie bei der Anaphylaxie. Dagegen fanden wir, daß die Resistenz der jodierten Tiere (welche also weniger Antitrypsingehalt zeigen) bedeutend vermindert war (9).

Der Mechanismus der Anaphylatoxinbildung.

Da sich die Fettsäuren und ihre Verbindung leicht absorbieren lassen und wir bewiesen haben, daß das antitrypsinfreie Serumeiweiß sich leicht spalten läßt und toxisch wirkt, untersuchten wir nun die Beeinflussung des Serumantitrypsins durch die verschiedenen adsorbierenden Substanzen, welche zur Anaphylatoxinbildung geeignet gefunden worden sind. Vergleicht man den Antitrypsingehalt des frischen Meerschweinchenserums und dasselbe Serum, nachdem es zur Anaphylatoxinbildung verarbeitet worden ist, sei es durch Digerieren mit Bakterien, spezifischen Präzipitaten, Agar, Stärke, Kaolin oder auch durch Toxinzusatz, so findet man stets eine bedeutende Herabsetzung des Antitrypsins. Diese läßt sich besonders durch Typhusbacillen, Agar und Stärke erzielen: durch Kaolin, durch Tuberkelbacillen und durch sonstige grampositive Bakterien läßt sich das Serumantitrypsin weniger beeinflussen.

Auch mit Diphtherietoxin wird das Antitrypsin herabgesetzt, wahrscheinlich wegen seiner lipoiden Affinität. Es ist wohl möglich, daß die Fettsäuren dieselbe Affinität für das Diphtherietoxin als für das Trypsin zeigen und es dadurch zu einer partiellen Absättigung kommt. Die Experimente Dolds und seiner Mitarbeiter (10) über desanaphylatoxierte Bakterien sind in dieser Beziehung von besonderem Interesse; mit derartig behandelten Bakterien war es uns nicht möglich, das Serumantitrypsin zu adsorbieren. Hirschfeld und Klinger (11) haben kürzlich, von vollständig anderen Beobachtungen ausgehend, die Vermutung ausgesprochen, daß es sich bei der Anaphylatoxinbildung um eine Lipoidadsorption handele, doch brachten sie dafür keinen experimentellen Beweis.

Von ganz entschiedener Bedeutung scheint uns der Befund, daß Bakterien (Typhus), welche nach Gebrauch zur Anaphylatoxinbildung getrocknet wurden, sich fast dreimal so resistent gegen die tryptische Verdauung als unbehandelte

Typhusbacillen erwiesen. Die Bacillen sind also bedeutend widerstandsfähiger durch die Serumbehandlung geworden. Solche Adsorptionsphänomene sollten nicht nur hier, sondern auch in manch anderen Phasen der Immunitätsforschung berücksichtigt werden, da sie einen wichtigen Unterschied zwischen den in vivo und in vitro bestehenden Verhältnissen darstellen (12).

Der akute Seifentod.

Es wäre vielleicht noch von Interesse, den akuten Seifentod kurz zu erwähnen, indem man es hier mit einem Shock zu tun hat, welcher den anaphylaktischen sehr ähnelt und somit die Seifen zu der schon langen Reihe Substanzen, welche den Tod durch anaphylaktische Symptome herbeiführen, einzureihen wären. Es wird im allgemeinen behauptet, daß Kolloide, welche akut töten, den Tod durch eine Herzthrombose verursachen und der Lungenblähung wird deshalb ein sekundärer Charakter zugeschrieben. Diesem gegenüber töten die Seifen bei einer M.L.D. ohne Gerinnung, unter dem Bilde eines typischen anaphylaktischen Shocks. Allerdings bei Einspritzung größerer Mengen Seifenlösung tritt auch eine Herzthrombose zutage, wie aus dem nachfolgenden Protokoll zu ersehen ist. In diesem Experimente wurde eine 1-proz. Lösung einer fast neutralen Oelseife gebraucht. Die gesättigten Seifen (z. B. eine Hydroxy-Palmitinseife), deren Löslichkeitsverhältnisse denjenigen der ungesättigten Seifen ähneln, erwiesen sich etwas weniger giftig, töteten aber auch mit typischem Shock und Sektionsbefund.

Protokoll II.

	Gewicht	Dosis ccm	Dosis in mg pro g Meerschw.	
1	320	3,0	0,097	Sofortige Sprünge. Tod in 2″. Lunge gebläht. Etwas Oedem. Rechte Herzkammer mit Gerinnsel gefüllt
2	310	2,5	0,08	Tod in 3″. Lunge stark gebläht.
3	350	2,0	0,057	Tod in 3″. Kein Gerinnsel. Typischer anaphylaktischer Befund
4	360	1,75	0,04	Keine Symptome
5	400	1,5	0,037	dgl.

Protokoll III.

Gesättigte Seife, 1-proz. Lösung.

	Gewicht	Dosis ccm	Dosis in mg pro g Meerschw.	
6	210	1,8	0,085	Plötzliche Krämpfe nach 3″. Herz schlägt stark. Kein Gerinnsel. Lunge gebläht. Blut flüssig.
7	500	3,0	0,06	Kratzt. Keine weiteren Symptome
8	340	1,5	0,044	Keine Symptome

Zusammenfassung.

1) Die Seifen der ungesättigten Fettsäuren hemmen die tryptischen und leukoproteolytischen Fermente. Die Hemmung steht in direktem Verhältnis zu der Jodzahl der Fettsäuren und deren Dispersitätszustand. Durch Absättigung der Fettsäuren wird die Hemmung aufgehoben.

2) Tuberkelbacillen enthalten ungesättigte Fettsäuren, welche verseift, bedeutende Hemmung erzielen.

3) Verkäste Gewebe enthalten ungesättigte Lipoide, welche die Verdauung hemmen. Durch Entfernung dieser Körper wird das verkäste Gewebe der Trypsinverdauung zugänglich. Der Aether oder Chloroformextrakt solcher Gewebe enthält die Hemmungskörper. Durch Jodbehandlung (Absättigung der ungesättigten Fettsäuren) wird das verkäste Material leichter verdaulich.

4) Das Serumantitrypsin besteht aus den ungesättigten Fettsäuren und deren Lipoidverbindungen im Serum. Es wird durch Aether oder Chloroform gelöst. Durch Verseifung des Aether- oder Chloroformrückstandes kann die antitryptische Hemmungskraft vollständig zurückerlangt werden. Die antitryptische Eigenschaft des Serums wird durch Jodabsättigung, durch Wasserstoffperoxid, sowie auch durch Einwirkung von Jodkalium und Jodnatrium aufgehoben.

5) Antitrypsinfreies Serum (gewonnen durch Chloroform- oder Aetherextraktion, durch Ansäuren und Filtrieren, durch Jodeinwirkung) wirkt höchst giftig. Schon partielle Beseitigung des Antitrypsins genügt, um toxische Effekte zu erzielen.

Der Tod wird durch drei Faktoren bedingt: a) eine sofortige intravaskuläre Gerinnung, b) eine Freilegung der Serumeiweißkörper, welche somit den Fermenten zugänglich werden, c) eine Bildung von toxischen Spaltprodukten aus Serumeiweiß durch Selbstverdauung. Hirudin und Na. citricum schützen nicht gegen das Serotoxin. Narkose schützt zum Teil. Vorbehandelte Tiere zeigen eine erhöhte Resistenz. Jodierte Tiere dagegen eine verminderte Resistenz.

6) Anaphxlatoxinbildung beruht auf Adsorption der Serumlipoide, mit dadurch folgender Freilegung des Serumeiweißes. Mit Komplement behandelte Bakterien werden nicht abgebaut, sondern zeigen erhöhte Resistenz gegen Trypsin — durch Adsorption des Serumantitrypsins bedingt.

7) Seifenlösungen töten Meerschweinchen mit akuten Symptomen die der Anaphylaxie ähneln.

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MALIGNANT MELANOTIC TUMOR, PRIMARY IN THE ADRENALS

ALWIN M. PAPPENHEIMER, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

The origin of melanotic tumors from tissues other than the skin and choroid has been considered highly questionable by so good an authority as Borst. Nevertheless, there have been reported from time to time, fairly well substantiated cases of melanotic growths arising from the adrenals. The number of cases in which the adrenal origin of the tumor is reasonably certain, is very small, and it seems desirable to place on record an additional example.

The patient, a man 50 years of age, entered the Presbyterian Hospital April 30, 1914.

CLINICAL HISTORY

Family History.—Negative.

Personal History.—Painter for 25 years. Gonorrhea and syphilis denied.

Past History.—Typhoid at 15 years. Painter's colic and blue line 12 years ago. Stopped painting 18 months and recovered. Otherwise well.

Present History.—Gradual onset four months ago with dull headache. For past year has had dull, cramp-like pains in stomach once or twice a day, not related to eating. No vomiting. A few days after headache began, had atack of fainting lasting 15 minutes. Then remained in bed because of weakness. For past three weeks legs have been weak. No wrist or foot drop. Headaches and stomach pains have continued irregularly. Bowels constipated. No disturbance of vision. Has lost some weight.

Physical Examination.—Well nourished. No dyspnea or cyanosis. Mentally clear. Head normal. Pupils small and equal, react normally. Arcus senilis. Upper teeth false, only stumps in lower jaw. Gums have receded and are soft. Marked bluish deposit in the gums at bases of teeth. Breath foul and tongue coated. Few large glands in submaxillary region. *Lungs* normal. *Heart:* Apex 4th space, 9 cm. to the left. No murmurs. *Blood Pressure* 142. *Abdomen:* Normal. Edge of liver indistinctly felt 3 cm. below costal margin. Spleen and kidneys not felt. All reflexes exaggerated. No œdema. No wrist- or foot-drop. *Superficial glands:* Mass in right axilla about 3.5 cm. × 2 cm. which feels like a mass of glands, is not tender, and freely movable. No other glands enlarged. There is peculiar blackish deposit in the skin below angle of right scapula. Lentigo over posterior chest.

May 1: Hgb. 75 per cent., W.B.C 12,800, Polys. 72 per cent.

May 2: Ophthalmoscopic examination negative. At 6:45 A.M. had many epileptic convulsions. Started with twitching in left arm and left side of face, then became unconscious and had clonic convulsive movements lasting 2–3 minutes. Very cyanotic. After convulsion perspired freely and was stuporous for about 2

hours, then irrational, complaining of something on back of his neck which should be turned off.

May 3: Phthalein test: Color appeared in 10 minutes. Total excretion 2 hrs. 21.7 per cent. Headache all day. Drowsy.

May 4: Lumbar puncture, pressure not increased. Leukocytes 2. Wassermann negative.

May 5: Blood urea 0.04 per cent. Lead present in urine. R.B.C. 4,438,000. No stippling. Hgb. 78 per cent.

May 6: Increasing stupor for 24 hours. Yesterday restless and severe headache. Involuntary discharge urine and feces.

May 7: Blood Wassermann negative. X-Ray of skull negative.

May 8: Phthalein test: Total 46.7 per cent. W.B.C. 20,500, Polys. 80 per cent., Lymphos. 19 per cent., Eosinophiles 3 per cent. No stippling of red cells. Lumbar puncture. Pressure not increased. Leukocytes 22, many r.b.c. Butyric acid test positive. Wassermann—0.1 c.c. Negative, 0.3 c.c. +, 0.5 c.c. ++. Lead present in urine. On May 7 temperature 102°, very stupid. Involuntary dejections. Muscles spastic.

May 14: May 12 very stupid with Cheyne-Stokes respirations. Brighter to-day, but irrational. No Cheyne-Stokes respirations. Muscles very spastic and reflexes exaggerated. Lumbar puncture: dry tap.

May 15: Lumbar puncture. Slightly turbid fluid under some increase of pressure.

Urine: Sp. Gr. 1013–1032, albumen absent three times, once a very faint trace. No casts.

Temperature: Between normal and 100°. Occasionally as high as 102°.

Blood Pressure: 94 160.

Autopsy May 16, 1914.

Body is that of an adult male measuring 166 cm. in length. The body is spare and poorly nourished. The visible mucous membranes are pale. Pupils equal. There is slight arcus senilis. There are no teeth in the upper gums. In the lower jaw, there are three incisors and one canine. The right middle incisor is

missing. The gums are retracted about the teeth and spongy. They show a dirty bluish discoloration. Liver mortis is present over the back and dependent portions. There are numerous light brown spots scattered over the shoulders and back. Beneath the angle of the right scapula there is a pigmented nævus. Rigor mortis is present in all extremities. The muscles of the limbs are not noticeably atrophied; those of the trunk are dry and a trifle pale.

The peritoneal cavity is free from fluid, the serosa glistening and smooth. The omentum contains a moderate amount of fat and is not adherent. The appendix is directed upward behind the cecum. There are fibrous adhesions between hepatic flexure and fundus of gall-bladder. Diaphragm reaches the 3d interspace on the right side, 4th interspace on the left side.

The pleural cavities are free from fluid and adhesions. The pericardium contains a few c.c. of clear yellow fluid. Adherent to the entire mediastinal surface of the pericardium in its upper portion, there is a rounded mass about the size of a walnut, which on section is found to consist of soft grayish-red tumor tissue.

Left Lung: The left lung weighs 520 grams, and is very voluminous. The pleura is shiny and smooth. The fissure between the upper and lower lobes extends only half way to the anterior border. On section the lung is everywhere well aerated, though very œdematous and mottled here and there with a few darker reddish areas. The bronchi are somewhat congested and contain frothy fluid. The bronchial lymph nodes are small and pigmented. The pulmonary artery shows a few yellowish patches. There are a few small, circumscribed, homogeneous white tumor nodules scattered throughout the substance of the lung.

Right Lung: The right lung weighs 600 grams. The pleura is normal. There is on this side also an anomalous lobulation, the fissure between the upper and middle lobes being represented merely by a shallow notch at the posterior border. On section, the appearance is the same as that of the left lung; the œdema perhaps is slightly more marked. Small tumor nodules are found in both upper and lower lobes. None of these exceed a few mm.

in size. The bronchial lymph nodes are enlarged and deeply pigmented. Cross section shows them to contain definite nodules of tumor tissue.

Heart: The heart weighs 310 grams. The epicardium is smooth. There is a moderate amount of epicardial fat. Along some of the smaller branches of the coronary arteries, there are seen a few minute flat whitish nodules. The right auricle is normal. The foramen ovale is closed. The tricuspid ring measures 10.5 cm. in circumference. The valves are normal. The pulmonary orifice measures 72 mm. in circumference. Wall of right ventricle measures 4–5 mm. in thickness. Left auricle is normal. The mitral ring measures 11 cm. in circumference. The valves are delicate. The aortic orifice measures 76 mm. in circumference. There are atheromatous patches on the aortic surface of the mitral valve. The aortic cusps are a little thickened at their bases. The edges are fenestrated, but delicate and freely movable. The left ventricle measures 12–14 mm. in thickness. The heart muscle is soft, a little brownish, and on section shows a few darker red areas, but no scarring. The coronary arteries are delicate and free from atheroma.

Liver: The liver weighs 2,150 grams and measures 28 × 21 × 9 cm. The surface is smooth, mottled yellowish red with scattered single lobules of bright yellow color. On section, the organ is of normal texture, with distinct lobules having a reddish center and paler grayish periphery. Near the falciform ligament, in the left lobe, there is a well-circumscribed nodule about 1.5 cm. in diameter, not much raised above the surface, composed of soft grayish yellow tissue with areas of opacity and hemorrhages. There is also a larger group of nodules situated in the substance of the right lobe near the hilus.

Gall Bladder: The gall bladder is distended with dark thin bile. The mucosa is smooth.

Spleen: The spleen weighs 275 grams and measures 14.5 × 9 × 4.5 cm. The capsule is roughened in places. On section, soft, with very large irregular gray follicles. The pulp is mottled with small hemorrhages.

Pancreas. The pancreas is of normal color and texture, and free throughout from tumor tissue. The splenic artery is wide and tortuous. The splenic vein is empty.

After removing the intestines and stomach, the adrenals are seen to be replaced by large rounded masses of tissue, conforming in a general way to the normal shape of the adrenals, and everywhere enclosed by a thin capsule, so that neither the kidneys nor the adjacent cellular tissue are invaded by the growth. The two large adrenal masses together with the kidneys, ureters, pelvic organs and great vessels of the thorax and abdomen were removed en masse.

The renal veins and vena cava are found to be free from thrombi or tumor masses. Several veins leading directly into the tumor masses were also opened and found to be empty.

Left Adrenal: The left adrenal forms a more or less globular mass which measures $7.5 \times 6 \times 3.5$ cm. It is very soft, almost fluctuating and on incising the mass, the tumor tissue bulges out of the tense capsule. It is extremely friable and soft, shows no distinct lobular structure. The surface of the cut section is in general grayish red, but variegated in as much as one can distinguish white areas having the consistence and color of brain tissue; with areas of cystic softening filled with fluid; looser grayish and grayish red portions and finally areas of hemorrhage. Just beneath the capsule there is a narrow strip of ochre-colored adrenal cortical tissue still recognizable, which can be traced over the anterior surface for a considerable distance. So far as can be determined, the tumor occupies therefore the medullary portion of the gland.

Right Adrenal: The right adrenal also is the seat of a tumor mass having the same general conformation and appearance as that described in the left. The mass on the right side measures $10 \times 6 \times 3$ cm. It is composed of the same sort of friable tumor tissue as the left adrenal, but is slightly more hemorrhagic.

Kidneys: The right kidney measures $10 \times 6 \times 3$ cm. The capsule is readily stripped. The surface is smooth and the cortex measures 5 mm. in thickness. Striations are distinct and

even. The color is a uniform reddish brown, the consistence firm but not hard. The vessels are not prominent.

Left Kidney: The left kidney measures $12 \times 5.5 \times 4$ cm. It resembles its mate in all respects.

Urinary Bladder: The mucous membrane is normal.

Prostate: The prostate is not enlarged, is symmetrical, of normal structure on section.

Testes: Slightly brownish, the tubules pull out with slight difficulty.

Œsophagus: Is normal in its lower portion, the upper part not examined.

Stomach: There is a circular constriction in the middle portion, due to muscular contraction. The mucosa is normal throughout, the folds marked only in the pyloric portion. There is a finger shaped diverticulum about 5 cm. in length and about the thickness of one's thumb, given off from the fundus, and directed upward and to the left. All three coats are apparently represented in the wall of the sac, but they are thinned out. The lining mucosa is not altered.

Intestines: The duodenum is normal. Small intestine: from the serous surface one can see and feel a number of irregular nodules scattered throughout the jejunum and the upper part of the ileum. These appear from without as irregular white areas, shining through the serosa and surrounded by injected venules. After opening the gut, the uppermost nodule is found about 5 cm. from the beginning of the jejunum. Here there is a submucous nodule about 2 cm. in diameter which projects in polypoid form into the lumen. It is entirely covered by intact mucosa, and on section appears as a circumscribed homogeneous soft white mass lying in the submucosa. From this point on, numerous similar metastatic nodules are found. The smallest ones appear merely as oval thickenings of the valvulæ. Large ones tend to become polypoid and one is distinctly pedunculated. The overlying mucous membrane is for the most part unaffected, but over some of the large tumors, there is distinct ulceration. The largest mass is found in the upper part of the ileum. It is oval

in shape, and extends about two thirds around the gut. It projects like a large button or raised disc into the intestine. The center shows a ragged crater-like excavation, and the surface is strikingly pigmented yellow and black.[1]

The appendix is 12 cm. in length. The lumen is patent and the mucosa normal up to within 2 cm. of the tip, where it becomes suddenly obliterated. The large intestine is normal throughout and is free from tumor nodules. The rectum also contains no new growth.

Lymph Nodes: The mesenteric lymph nodes are not much enlarged, but some of them on section contain circumscribed tumor nodules. The retroperitoneal and hypogastric lymph nodes are also the seat of metastatic deposits, but are not much enlarged. The lymph glands at the bifurcation of the trachea form a packet of considerable size. On section they also contain circumscribed tumor nodules, standing out in sharp contrast to the anthracotic lymphoid tissue.

The organs of the neck, head and spinal cord could not be examined, permission being withheld.

Right Adrenal: Transection after hardening shows a continuous shell of cortex surrounding the entire tumor mass. This is thinned out to 1 mm. or less on the anterior surface, and is thickest at the upper and lower poles, where one may distinguish portions of the pigmented zona reticularis.

Left Adrenal: The left adrenal is also enclosed in a very thin shell of cortical tissue.

MICROSCOPICAL EXAMINATION

Tumor Tissue: With variations to be noted, the tumor tissue shows the same structure in the original growth and in the metastases. It is composed for the most part of round or polyhedral cells of rather large size having a round, occasionally slightly oval or indented nucleus which stains well and shows a finely distributed delicate chromatin network without a large or distinct nu-

[1] A close examination of the intestinal nodules in the hardened museum specimen shows distinct patches of a grayish color scattered through the cream colored tumor tissue.

cleolus. In many places are found cells containing two or three nuclei and in a few places larger conglomerations of nuclei with a common protoplasmic syncytium. These giant cells may contain ten or twelve closely clustered nuclei. The cytoplasm in some places is very indefinite, pale, and finely granular. In other places the cell outline is more sharply defined. It is usually spheroidal, not polygonal, but not infrequently is drawn out into a short spindle.

In the intestinal nodules the cells are somewhat larger and disposed in a sort of alveolar arrangement. Many of them contain a fine brownish granular pigment, the amount of which and the color vary in different cells. This pigment gives no iron reaction, is not removed by lipoid solvents, and resists decolorization by 5 per cent sulphuric acid and by strong alkalies. With polychrome methylene blue, it takes a deep greenish-blue color. The nuclei of these pigmented cells show curious degenerative changes. Many of them are shrunken and pyknotic. In others the central portion of the nucleus is clear and the chromatin lies against the nuclear membrane giving a ring or signet appearance. In some cells the degeneration is more extreme and the chromatin fragmented into deeply staining particles which are found also free between the intact cells or as inclusions within other cells. In the intestinal nodules there are scattered eosinophilic leukocytes between the tumor cells. These also show the nuclear pyknosis and fragmentation. The arrangement of the tumor cells is highly irregular. In many places they are loosely aggregated and not separated by a definite stroma, so far as can be judged in hematoxylin eosin stains. They may form nests of solid alveoli separated by loose strands of connective tissue. In still other places the individual cells are separated by an ill-defined finely fibrillar staining substance. In still other places, and this is especially conspicuous in the original tumor, the cells are grouped in a more or less radial fashion about thin-walled blood vessels. Between these mantles of intact tumor cells there are often extensive fresh hemorrhages. Large areas of necrosis, involving not only the tumor cells but the blood vessels and stroma as well, are present in

many of the nodules, especially in the large metastatic growths in the bronchial lymph nodes. In other places the tumor tissue is rarefied by collections of fluid coagulum. Mitoses occur with moderate frequency.

Lungs: The capillaries are very congested and somewhat tortuous. The alveoli are for the most part empty but some of them do contain a loose exudate composed principally of polynuclear cells and of a little granular coagulum. The tumor nodules are well circumscribed, show no definite relation to blood vessels and are free from necrosis. With the immersion, isolated cells show diffuse brownish pigmentation.

Liver: Section passes through a large tumor nodule in which the tumor cells show a distinct perithelial arrangement with intervening areas of necrosis. It is surrounded by a dense fibrous capsule which sends septa between different portions of the tumor tissue. About the tumor nodule there is a tangential compression of the liver cells and a very striking dilatation of the capillaries with formation of large sinuses. There is also a definite œdema of the liver tissue and the accumulation of many wandering cells principally mononuclear but occasionally eosinophiles both in the distended capillaries and outside of the capillary wall between it and the compressed liver cells. Some of the liver columns show quite distinct accumulation of brownish granular pigment.

Spleen: The sinuses are very distinct from the rather fibrous pulp and are well filled with red cells. There are a few irregular hemorrhages. The Malpighian bodies are moderate sized, composed throughout of cells of the small lymphoid type. The trabeculæ and the blood vessels show nothing of note.

Kidney: There is a well-marked congestion of all the capillaries including those of the tufts. The glomeruli are normal. The epithelium of the convoluted tubules is somewhat swollen, with a distinct granulation of the protoplasm. The blood vessels show a slight increase in the adventitious coat, but are not otherwise altered.

Intestinal Nodules: Sections through various intestinal nodules show essentially the same picture. The tumor growths are

found in the submucosa or in the loose tissue of the mucosa itself. The muscular coat in the larger growth is compressed but not infiltrated. One of the nodules has distinctly a polypoid form, and the mucous membrane continues for a considerable distance on the inferior surface of the polyp, but is necrotic over its convex portion.

Bronchial Lymph Glands: The bronchial lymph nodes are extensively replaced by tumor tissue. They show in addition a few old tuberculous foci. In the capsule of the gland are found irregular brownish concretions in some places surrounded by foreign body giant cells.

Prostate: The ducts are dilated and filled in many places with desquamating epithelium which is heaped up. In some of the tubules the heaping up of the epithelium is so marked that the lumen is practically obliterated. There is also a good deal of papillary infolding.

Myocardium: Aside from slight œdema and fragmentation of the fibers, is normal.

Stomach: Is normal.

Anatomical Diagnosis: Primary malignant tumor of adrenals, with metastases in liver, lungs, bronchial, peritracheal, retroperitoneal and mesenteric lymph-nodes, and in small intestine. Œdema of lungs. Brown atrophy of heart. Diverticulum of stomach. Localized chronic appendicitis-with obliteration. Pigmented nævus.

DISCUSSION

The primary origin of the tumor from the adrenals in this case seems beyond question. In view of the ophthalmoscopic examination, it is unlikely that there existed a choroidal tumor, and there were no cutaneous growths aside from the small pigmented nævus, which showed no histological signs of malignancy.

In spite of the absence of pigment in the adrenal tumors themselves, the melanotic character of the growth is perfectly evident from a study of the intestinal metastases, in some of which the pigment is very abundant. One finds also occasional pigmented cells in the pulmonary and lymphatic nodules, but only after careful search.

The chemical reactions of the pigment, so far as they were studied, agree with those given by the normal pigment of the reticular zone. Lucksch[1] has studied the pigment in benign pigmented adenomata of the adrenal cortex, and obtained similar reactions. The crude tests which we have applied are at least sufficient to exclude an accidental pigmentation from previous hemorrhages, and to suggest that the pigment is elaborated by cells derived from the adrenal cortex.

The previously reported cases of melanotic tumors of adrenal origin are too few to justify an extended analysis. In four of the nine reported cases which I have been able to find, the data are too incomplete to make the adrenal origin of the tumors certain. Thus in a case mentioned by Orth,[2] and described by Döderlein,[3] there is no record of the eyes or skin, and no microscopical examination. Another case, reported by Davidsohn,[4] was later found by Marchand to have had a tumor of the choroid. The data in two other cases—one of Orth's and one published by Reimann[5]—are also too meager to justify conclusions.

The cases of Schweikert,[6] Davidsohn[7] (Case 2), Schmidt,[8] Schum,[9] and Molnar[10] are well-reported, and include references to the condition of the eyes. The tumors have all occurred in later life (except the case recorded by Molnar in a man of twenty-nine), and four of the five have been in males. In all the cases, there were secondary growths in the gastro-intestinal tract, particularly, as in our case, in the jejunum. The brain, lungs, lymph-glands seem also to be especially disposed, the liver metastases, in contrast with the general rule in melanotic tumors, few or entirely wanting. Cutaneous or subcutaneous nodules were usually present, and two of the cases showed growths in the bones.

Schum's case is interesting in that the original adrenal tumor was pigment-free, and only the metastases pigmented.

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THE EFFECT OF PENETRATING RAYS UPON EXPERIMENTAL TUBERCULOSIS

LEO KESSEL, M.D., AND M. J. SITTENFIELD, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

I

In 1896 Lortet and Genoud[1] first mentioned the influence of X-Rays upon experimental tuberculosis. They used the simple X-Ray apparatus in vogue at that time and histological studies were not made. Rieder[2] in 1899 found that a certain inhibitory process was exerted upon local tuberculosis when the animals were exposed to X-Rays. Muhsam[3] inoculated guinea-pigs subcutaneously in the inguinal region and exposed this superficial situation to the X-Ray. At the point of inoculation he noted in the rayed animals limitation and encapsulation of the tuberculous process. The average length of life of control animals was 53 days and of experimental animals 67 days. Kupferle[4] later inoculated rabbits intravenously, produced tuberculosis and exposed these animals to penetrating rays; although the ray dosage appears to have been inadequate, he succeeded in producing a fibrosis of the tubercles in the lungs of the rayed animals.

Our experiments were undertaken with the view of determining whether the pathological changes in tuberculous animals were modified by the penetrating rays. Accurately weighed quantities of dried human tubercle bacilli, which had grown for 3½ weeks on Dorset's egg medium, were suspended in sterile salt solution and used for inoculation into guinea-pigs. The pigs

varied in weight from 310 to 670 grams. The control and experimental animals in each case were approximately equal in weight.

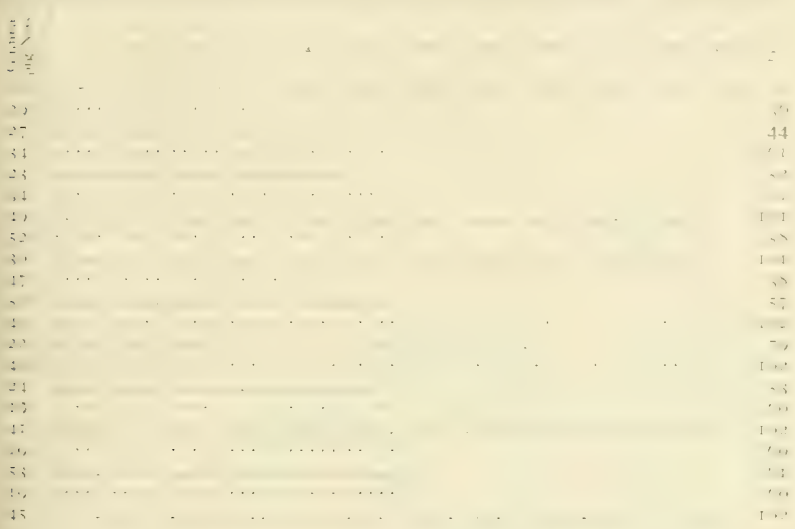
Fourteen pigs were injected intravenously with 1/100 to 1/200 mg. of the dried tubercle bacilli in salt solution, while 13 were injected intraperitoneally with doses ranging from 1/100 to 1/10 mg. of the same culture. On the 12th day after inoculation guinea-pig No. 20 died, and autopsy revealed extensive tuberculous processes throughout all the organs and tubercle bacilli were demonstrated in the spleen. Of the remaining 26 animals, 7 controls died before the experiments were well under way and 3 experimental animals died after one exposure to the penetrating rays, necessitating the use, in some instances, of one control to 3 experimental animals. On the 32d day following inoculation exposures to the penetrating rays were made. The Gauss X-Ray apparatus was used and the rays applied to the dorsal, ventral and lateral surfaces over a period of 20 days, each animal receiving a dose of 8–10 X to each surface, in cross fire fashion, every 3d day. The focus distance was 20 cm., the penetration 10 B, and the length of exposure 6 minutes to each surface. The total dosage received ranged from 70 to 183 Kienbock units. The animals were bright and well and no external reaction was observed in any of them. The control animals lived 36, 38, 58, 60, 61 and 102 days respectively, the rayed animals lived 44, 52, 53, 57, 61, 79, 101, 101, 102 and 102 days respectively. The total length of life of 6 control animals was 362 days, a mean average of 60⅓ days. The total length of life of 10 experimental animals was 752 days, a mean average of 75⅕ days. Thus the experimental animals outlived the controls on an average of 15 days per animal.

The chart of duration of life of both sets of animals is given below:

The animals were autopsied in almost every case within 24 hours after death. Macroscopically they all showed tuberculous lesions in lungs, liver, spleen and lymph nodes.

The lungs of the controls upon microscopic section revealed in-

tense congestion, hemorrhages into the alveoli, tuberculous pneumonia and numerous tubercles with central caseation. In the rayed animals on the other hand, we observed very few tubercles with cheesy centers, and many of the tubercles showed fibrous encapsulation.



..... = control animals.
= treated animals.

In the liver of the control animal large areas of complete necrosis were seen, some of these areas involving an entire lobule. There were also numerous tubercles, originating in the portal spaces, presenting the picture of a progressive lesion with few epithelioid or giant cells. A few of the tubercles in the liver, notably those seen in sections from guinea pig No. 40, showed a certain amount of fibrosis. The rayed animals with the exception of No. 50 (which received 10 times the dose of the others) revealed fewer and smaller areas of necrosis; as a rule, the tubercles were miliary in character and frequently were surrounded by fibrous tissue; many appeared healed, and in some livers there were large atrophic areas about the portal spaces with connective tissue replacement.

In the spleen of some of the control animals very little normal tissue was visible; masses of necrotic and caseous tissue replaced the follicles. The sinuses were very wide and separated by little pulp tissue. With the exception of guinea pig No. 40 the tubercles in the spleen showed little fibrosis. In the rayed animals many tubercles in the spleen were completely fibrosed and few of them showed small caseous centers.

The lymph nodes of the control animals contained large areas of caseation and some small areas of fibrosis, while the nodes of the rayed animals contained a few tubercles with central caseation and many completely fibrosed tubercles.

From these experiments the following conclusions may be drawn:

1. Guinea-pigs in which experimental tuberculosis has been produced and which have subsequently been exposed to penetrating rays, live longer than their controls.

2. Caseation is less evident in the tuberculous lesions of the rayed animals, and fibrosis about the individual tubercles is more marked.

These conclusions may appear more significant when consideration is taken of the fact that radiation was instituted at an unfavorable stage of the disease, when the tuberculous process was widespread and had undergone caseation. Further experiments are being pursued to study the effect of radiation in the earlier stages of tuberculosis, and to determine the period at which these lesions are most favorably influenced. The details and protocols of the entire study are reserved for future publication.

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The Therapeutic Action of Iodin

JAMES W. JOBLING, M.D.
AND
WILLIAM PETERSEN, M.D.
NEW YORK

THE THERAPEUTIC ACTION OF IODIN*

JAMES W. JOBLING, M.D., AND WILLIAM PETERSEN, M.D.
NEW YORK

Though iodin in the form of inorganic iodids or as organic compounds has been used for years, and is one of the most important drugs in the Pharmacopeia, little is known concerning its action in the body. This lack of knowledge applies particularly to its effect in causing the absorption of necrotic material such as is found in gummas.

The results obtained with the iodids have been variously ascribed to their influence in the general metabolism of the body; to their supposed action in causing a fall in blood-pressure; to a change in the viscosity of the blood; to a lymphocytosis; to an increase in the activity of the lymphatics, and to the oxidizing properties of the nascent iodin.

Binz[1] and Hinz[2] believed its action to be due to the oxidizing properties of the nascent iodin, which, according to Hinz, renders the blood-vessels more permeable and makes the leukocytes more active. Romberg[3] suggests that its action may be due to some change in the blood, and Müller and Inada[4] state that this change consists in an alteration of the viscosity. Determann,[5] however, denies that there is a change in the viscosity of the blood following the administration of iodids.

Various authors have ascribed the action of iodin to its property of causing a fall in blood-pressure, but Stockman and Charteris[6] state that there is no fall in blood-pressure when iodids are given. Lehndorf,[7] however, in a recent paper states that iodids do cause a fall of the blood-pressure, and believes that the lowered blood-pressure aids healing by permitting a better circulation of blood through the diseased area.

Much work has been done on the absorption, distribution and excretion of iodin; we shall, however, refer only briefly to this. All who have investigated the subject have found that the inorganic

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* From the Department of Pathology of the College of Physicians and Surgeons, Columbia University.

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iodids are absorbed very rapidly, and in the main are excreted as inorganic compounds, while the organic compounds, especially those in which the iodin is combined with fatty acids, as in iodipin, are absorbed and excreted more slowly. According to Winternitz,[8] iodipin is absorbed from the intestinal tract as iodized fatty acids, and by oxidation of these the iodin is split off and is excreted principally in the form of inorganic iodids. Wells[9] concludes from his work that when iodipin is injected into the subcutaneous tissues it is carried in the blood chiefly as inorganic iodids. McLean[10] made a study of the distribution of iodin in the body of rabbits which had received several doses of potassium iodid, and found that the lipoid fractions of the tissues contained 32 per cent. and the water-soluble fraction 67 per cent. of the iodin. The extracted protein contained no iodin. The older view that iodin combines with the proteins of the body has been disproved by McLean and other observers. We believe we are justified in assuming that a large part of the iodin combines with the lipoids, as the work of McLean and Winternitz indicates that such organic compounds, whether given as such or formed in the body, are in turn readily oxidized, and the iodin excreted chiefly as inorganic compounds. Bröking[11] has shown that iodin administered as iodized fats or fatty acids is not excreted so rapidly as when given in the form of potassium iodid. This is to be expected, as absorption would be less rapid, and new compounds must be formed before it can be excreted, whereas potassium iodid is more readily absorbed and excreted as such.

Reference books on pharmacology and therapeutics afford but little information on the action of iodin. Forschheimer[12] states that iodin stimulates the activity of the lymphatics and increases the energy of the nutritive processes, and thus promotes the absorption of disease products. Schmiedeberg[13] states that iodin does not exert a specific action on any organ, but causes a change in metabolism and in nutritive processes in general.

In the present work we have confined our investigations of the action of iodin to its influence on the antitrypsin of the blood and tissues, as we believe that the antitrypsin is the most important factor in preventing the resolution of necrotic tissues such as are found in infarcts, and in the caseous areas in syphilis and tuberculosis.

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8. Winternitz, H.: München. med. Wchnschr., 1903, i, 1241.
9. Wells, H.: Ztschr. f. physiol. Chem., 1905, lxv, 412.
10. McLean, F. C.: Organic Iodin Preparations, Their Pharmacology and Therapeutic Value, The Archives Int. Med., November, 1912, p. 505.
11. Bröking, E.: Ztschr. f. exper. Path. u. Pharmakol., 1911, viii, 125.
12. Forschheimer, F.: Therapeusis Int. Dis., 1913, ii, 404.
13. Schmiedeberg, O.: Grundriss d. Pharmakologie, 1913, Ed. 7, p. 449.

ANTITRYPSIN

In a recent publication[14] we reported that the ferment-inhibiting action of serum is due to the presence of compounds of the unsaturated fatty acids. The ease with which they can be extracted by chloroform or ether indicates that they are either free acids, or that they are combined with lipoids. The influence of chloroform on the antitrypsin of serum and the rapidity with which it acts is shown in Chart 1. In this experiment two volumes of chloroform were added to fresh serum and the mixture was placed in the incubator for one hour. During this time it was shaken thoroughly at short intervals. At fifteen-minute intervals some of the serum was removed and tested for its ferment-inhibiting properties. In separating the serum from the chloroform it is necessary to centrifuge at high speed for about five to ten minutes,

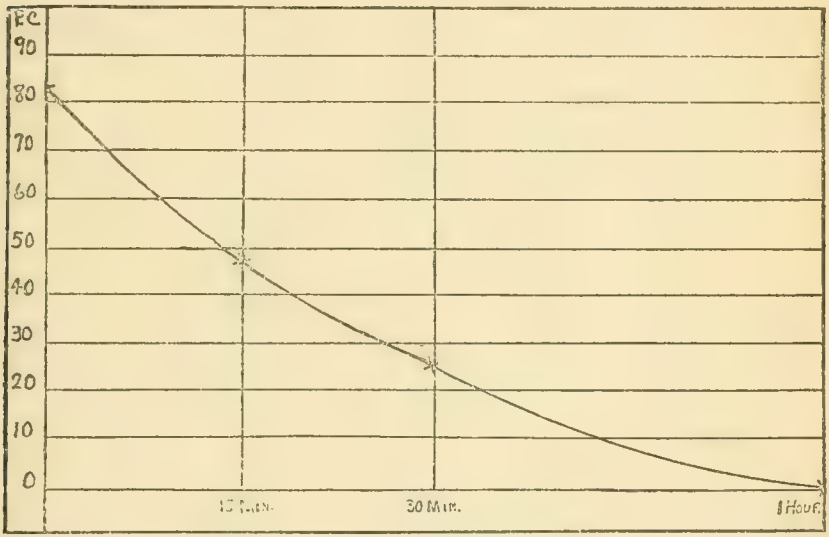

Chart 1.—Rate of removal of serum antitrypsin by chloroform extraction at 37 C. (98.6 F.).

and then to filter several times through coarse filter-paper until the serum is quite clear and all the chloroform evaporated. This experiment demonstrates that the ferment-inhibiting action of the serum is almost wholly lost after sixty minutes' incubation with chloroform.

That the inhibiting action is due to fatty acids is shown by the following experiment, the results of which are shown in Chart 2.

Ether was added to fresh dog-serum, and the mixture was allowed to stand at room temperature for two days. The ether was then removed, evaporated to dryness and the substance remaining saponified

14. Jobling, J., and Petersen, W.: Jour. Exper. Med., 1914, xix, 459.

by means of sodium alcoholate. After evaporating to dryness, the soaps were dissolved in water, the acids liberated in the usual manner, taken up in ether and resaponified. These soaps and some of the untreated serum were then tested to find out their ferment-inhibiting action.

The chart shows that the soaps prepared from the ether extract cause nearly as much inhibition as the untreated serum. Subsequent experiments showed that the chloroform and ether extracts lost their ferment-inhibiting action if they were first treated with iodin, which indicates that the active agents were the unsaturated fatty acids.

The demonstration that the antitryptic action of the blood and of tuberculous caseous matter is due to the presence of unsaturated fatty acids suggested to us the possibility that the action of iodin in the body

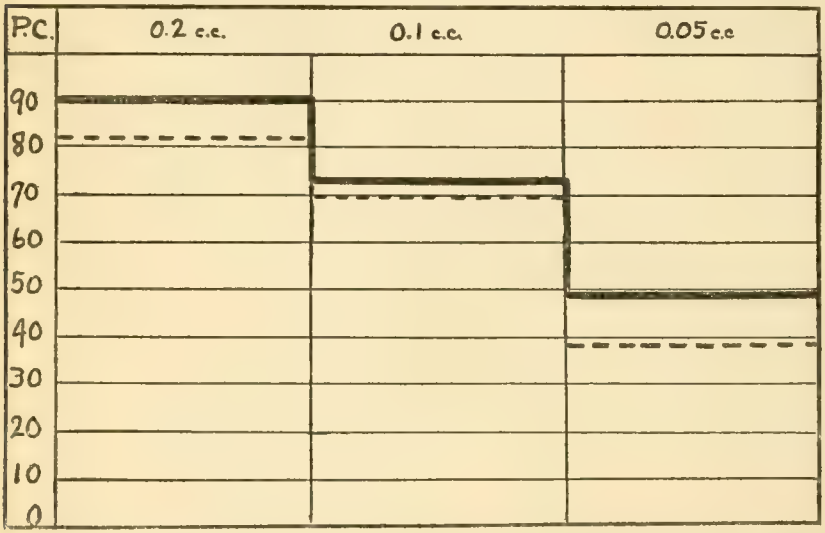

Chart 2.—Antitryptic effect of saponified ether extract of normal serum. Solid line, inhibition of normal serum; broken line, inhibition of saponified extract.

may be due to a combination with the fatty acids, thus causing a neutralization of the activity of these substances as ferment-inhibiting agents. If this supposition proved true, the neutralization of the activity of these agents should lower the antienzyme strength or the blood-tissues in general and permit the removal of dead tissues by autolysis.

The next experiment was made to determine the influence of potassium iodid on the antitrypsin of guinea-pig serum.

Fresh guinea-pig serum was mixed with potassium iodid in the proportion of 0.2 gm. of the iodid to 1 c.c. of the serum, and the mix-

ture placed in the incubator over night. Some of the untreated serum was used as a control. Chart 3 shows the results of this experiment.

The chart shows the great decrease in antitryptic strength of the serum after treatment with potassium iodid. Similar results were obtained when the serum was treated with hydrogen dioxid. The foregoing observations led us to study the antitryptic strength of the serum of patients to whom iodids were being given. It seemed probable, especially from the results obtained by McLean, that some of the iodin would combine with the unsaturated fatty acids of the body, and as a result lower the antitryptic strength of the serum and of the tissues in general. This decrease in antitrypsin would permit the ferments normally present in the necrotic areas to become active, and thus hasten autolysis.

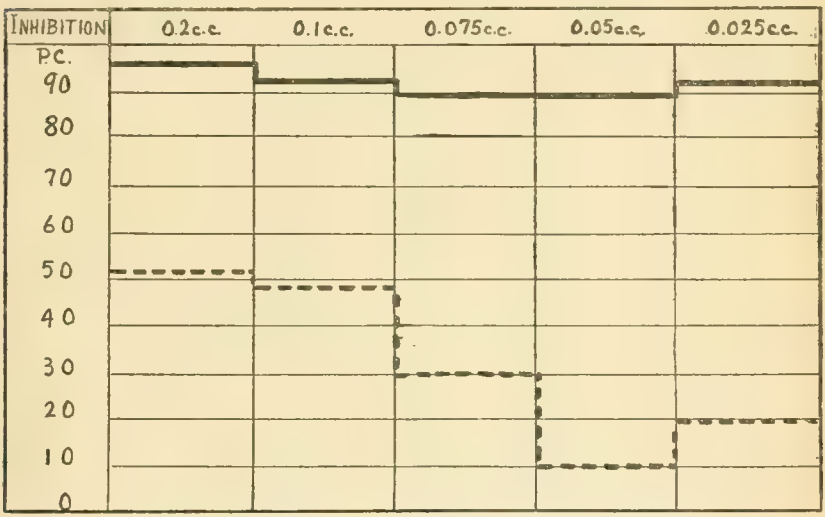

Chart 3.—Effect of potassium iodid on antitrypsin of guinea-pig serum. Solid line, normal serum; broken line, normal serum with potassium iodid incubated over night.

We are greatly indebted to Drs. J. Fordyce, W. McMurty and C. Sharpe for furnishing us with the clinical material used in this study, and also for regulating the dosage of iodids and determining the clinical condition of the patients while the work was progressing. Thirteen cases were studied, eleven of which were syphilitics. In each instance the blood was tested before iodids were given. The second test was made when the patient was receiving about 100 grains a day, and the third when he had reached the limit of tolerance. We were unable to carry out this plan in all cases, as the patients did not always follow directions.

In making the tests the trypsin was mixed with the various dilutions of serum and incubated thirty minutes. Two c.c. of a 1 per cent. casein solution was then added to each tube, and the mixtures were incubated one hour. The contents of the tubes were then acidified with a mixture containing 10 per cent. glacial acetic acid and 20 per cent. sodium chlorid and the tubes placed in boiling water for from five to ten minutes. The mixtures were then filtered through kaolin, and non-coagulable nitrogen was determined by the method recommended by Folin and Denis.[15] The results are given in percentage of total control digestion. The trypsin used in this work was prepared according to the method described in a previous paper,[16] and made into solution when required.

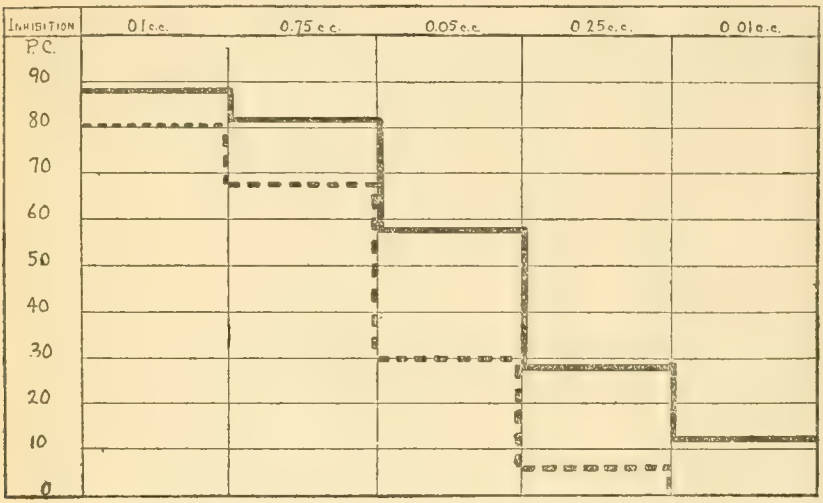

Chart 4.—Average of human cases on potassium iodid (110 gr.).

Chart 4 gives the average of the antitryptic strength of the serums of the thirteen cases before and after using iodids. The black line represents the strength of the serums before iodids were administered, and the dotted line the average strength of the serums when the patients were taking about 110 grains a day. The action of the iodids is best seen in the dilutions beginning with 0.05 c.c. of serum, though some effect is observed in the more concentrated solutions.

This series of cases is not large, but sufficiently so to show that iodids administered to human beings cause a very considerable reduction in the antitryptic activity of the blood. The difference is not so evident when larger amounts of serum are used, as here there is an

15. Folin, O., and Denis, W.: Jour. Biol. Chem., 1911-12, xi, 527.
16. Jobling, J., and Petersen, W.: Jour. Exper. Med., 1914, xix, 239.

excess of the enzyme-inhibiting agent over that necessary to neutralize the amount of trypsin used; but in great dilutions the difference is very obvious.

It is of interest to note that in the two cases of iodism the antiferment was much higher than it had been during the course of treatment. This increase in the antitrypsin may be due to the failure of the iodin to combine with the unsaturated fatty acids, or to an increase of lipoids following the destruction and disintegration of cells as a result of the toxic action of the iodin. It is probably due to the latter factor. It is possible that when large doses of iodids are given there may be localized areas in which the antiferments are completely neutralized; with a consequent destruction of cells by the ferments present and a liberation of lipoids. Thus an increase in antitrypsin is found in almost every case in which a protein intoxication causing cellular injury with mobilization of lipoids is induced experimentally, as after anaphylactic shock, after burns, after serotoxin and anaphylatoxin injection. The iodin may also combine with and neutralize the protective substances in the cell wall and thus permit the ferments to act on them. This may partially explain the toxic action of iodin.

We have recently shown[17] that serum deprived of its antitrypsin becomes toxic for the species from which it is obtained. If guinea-pig serum is shaken thoroughly with two volumes of chloroform and then placed in the incubator for one hour, it becomes so toxic that 2 or 3 c.c. injected intravenously will kill a guinea-pig weighing from 250 to 300 gm. If the mixture of serum and chloroform is permitted to remain in the incubator twenty-four hours, 0.3 c.c. will frequently kill a guinea-pig of this weight. The toxicity of serum treated in this manner is due to the fact that the chloroform removes the antitrypsin, and when this occurs, the ferments, which are normally present, act on the unprotected serum proteins and produce the toxins. Autolysis occurs to a very slight degree during the first hour in the incubator, but after twenty-four hours there is a considerable increase in non-coagulable nitrogen. Up to a certain point the toxicity increases in proportion to the degree of autolysis, but it then decreases rapidly, owing to further cleavage of the toxic substances. Similar results may be obtained with any agent which absorbs the antitrypsin. Thus we have found that kaolin, agar, certain toxins and bacteria act in the same manner, though to a less degree. The antitryptic action of the serum may be destroyed without removing the lipoids. Certain oxidizing agents, such as iodin, saturate the unsaturated carbon bonds of the fatty acids, and in this way destroy their antitryptic action, and render the serums toxic. In our study of these toxins we found that the

17. Jobling, J., and Petersen, W.: Jour. Exper. Med., 1914, xix, 480.

toxicity of the serums treated by the different methods depended on the degree to which the antitrypsin had been removed. Autolysis of the serum also depends on this factor.

If the action of the iodin in causing absorption of necrotic material is due to the general lowering of the antitrypsin, large doses should cause an increase in the nitrogen output owing to the increase of proteolysis. The results of a number of experiments with dogs indicate that the iodids do increase the nitrogen output, but these experiments are not sufficiently advanced to enable us to report on them at this time.

TUBERCULOSIS

There is a general impression among clinicians that iodids are harmful in tuberculosis, as they promote the spread of the infection by causing a softening of the tubercles, and also interfere with connective-tissue formation. On the other hand, L. Bondreau[18] regards iodin as a specific in tuberculosis. He begins treatment with small amounts of the tincture (the French tincture contains 8.5 per cent. of iodin in 95 per cent. alcohol), and increases the dose to 100 drops a day. He states that some of the patients pushed the doses themselves to 200 or 300 drops a day, and one patient took 400 drops a day without the slightest inconvenience.

Loeb and Michaud[19] found that when iodin compounds were injected into the body tuberculous areas took up more iodin than the other tissues. They observed that a tuberculous rabbit's eye contained about twice the amount of iodin present in the normal one, and that caseous lymph-glands of guinea-pigs contained more iodin than any of the normal organs. Wells and Hedenberg[20] repeated the work of Loeb and Michaud, but in addition made experiments to determine the power of necrotic tissue in general to take up and hold iodin. The authors found that tuberculous lymph-glands do take up relatively more iodin from the blood than from the liver, spleen and other glands of the same animal. When the caseous material was abundant enough to permit of separation from the rest of the gland substance, it was found to contain more iodin than did the non-caseous portion. An important part of their work was the demonstration that iodin was taken up equally well by necrotic tissues and exudates produced experimentally. They conclude that the large amount of iodin present in necrotic tissues, whether tuberculous or otherwise, is dependent on

18. Bondreau, L.: Jour. de méd. de Bordeaux, 1914, lxxxv, 1.
19. Loeb, O., and Michaud: Biochem. Ztschr., 1907, iii, 301.
20. Wells, H., and Hedenberg, O.: Jour. Infect. Dis., 1912, xi, 349.

purely physical conditions, and not on a chemical attraction or physical absorption.[21]

Since the reports of Mosetig and Moorhof,[22] iodin in the form of iodoform has been used extensively in the treatment of local tuberculous conditions. Bruns and Nauwerck[23] found that the walls of abscesses treated in this manner were invaded by leukocytes, but they ascribed the disappearance of tubercle bacilli in the inflated tissues to the germicidal action of the iodoform. Heyn and Rovsig[24] and others, however, showed that iodoform has no germicidal action on tubercle bacilli. In 1904 Heile[25] demonstrated the presence of proteolytic ferments in the pus of tuberculous abscesses treated with iodoform, and ascribed the curative action of the iodoform to the accumulation of leukocytes. Weil[26] injected iodoform-glycerin mixtures subcutaneously into both healthy and tuberculous individuals in order to study their influence on the blood. Immediately following the injection there was an increase in the polynuclear cells and a decrease in the number of lymphocytes, but this was soon followed by an increase in the lymphocytes and a decrease in the polynuclears. Most of the investigators believe that the active component of the iodoform is the iodin, and as it is known that the administration of iodin causes an increase in the number of lymphocytes in the blood, Gianasso[27] and others ascribe its action to these cells. According to Bartel and Neuman,[28] the lymphocytes contain substances which neutralize the toxins of the tubercle bacilli, and Bergell[29] believes that this action of the lymphocytes is due to their containing lipase which acts on the neutral fat of the tubercle bacilli. Rothschild[30] believes that iodin stimulates phagocytosis as he found that a much larger number of the tubercle bacilli in the sputum of tuberculous patients receiving iodids were intracellular.

In our work[31] on the antiferments contained in tubercle bacilli and in tuberculous caseous material, we found that the enzyme inhibiting

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21. In a recent paper Lewis and Krauss (Jour. Biol. Chem., 1914, xviii, 313) have demonstrated that tuberculous tissue from animals which were never under iodin treatment may contain very appreciable amounts of iodin, thus throwing some doubt on the conclusions reached by Loeb and Michaud and by Wells and Hedenberg.

22. Mosetig and Moorhof: Wien. med. Wchnschr., 1880, xxx, 1174.

23. Bruns, P., and Nauwerck, C.: Beitr. z. klin. Chir., 1887, iii, 133.

24. Heyn, C., and Rovsig, T.: Fortschr. d. Med., 1887, v, 258.

25. Heile: Ztschr. f. klin. Med., 1904, lv, 508.

26. Weil, W.: Ztschr. f. Chemotherap., 1913, Orig., i, 412.

27. Gianasso: Referred to in München. med. Wchnschr., 1905, lii, 1990.

28. Bartel, J., and Neuman, W.: Centralbl. f. Bakteriol., 1905-1906, Orig., xl, 723.

29. Bergell: München. med. Wchnschr., 1910, lvii, 1683.

30. Rothschild, W.: Deutsch. med. Wchnschr., 1913, xxxix, 404.

31. Jobling, J., and Petersen, W.: Jour. Exper. Med., 1914, xix, 251; ibid., 1914, xix, 383.

action was due to the unsaturated fatty acid radicles of the lipoids. At first we believed that this action was due entirely to the soaps of the unsaturated fatty acids, but subsequent work showed that a large portion of the enzyme-inhibiting agents could be removed by extracting with chloroform. The substances contained in the chloroform extracts are not active as antienzymes when suspended in salt solution, as they are insoluble; but the sodium soaps prepared from these acids are active, and we found that their activity was in proportion to the degree of their unsaturation.

In our study of soaps as enzyme-inhibiting agents[16] we found that certain unsaturated fatty acids in the form of soaps were very active as antiferments, but that this action was lost if the acids were first saturated with iodin. These experiments were repeated with the chloroform extracts of tuberculous caseous material and with unextracted caseous material, with the result that the soaps prepared from the chloroform extracts became inactive, and the caseous matter which, previous to treatment with iodin, had not been acted on by trypsin, became readily attacked. The loss of antienzyme action cannot be attributed to any activating influence on trypsin, as iodin inhibits the action of this ferment.

Thus we have definite evidence of the action of the iodids on tuberculous tissue, and the explanation of the well-known clinical observation that iodids cause tubercle bacilli to appear in the sputum of patients with pulmonary tuberculosis, though they were previously absent. When iodids are taken into the tissues a portion of the iodin is liberated and combines with the unsaturated carbon atoms of the fatty acids. As soon as this occurs in necrotic tissue to a degree sufficient to lower or remove the antiferment action, autolysis ensues, as in most instances ferments are present. That the inorganic iodids diffuse readily throughout caseous matter and are thus in a position to furnish the iodin has been shown by Loeb and Michaud, and Wells and Hedenberg.

Tubercles and caseous areas may be present in the lung, but unless they open into the air passages tubercle bacilli may be absent in the sputum. If iodids are now given, the caseous matter begins to soften, and is more likely to rupture into the bronchi, and the bacilli then discharged in the sputum. A somewhat similar explanation may be advanced to explain the softening and rupture of caseous areas, as in lymph-glands, when they are secondarily invaded by streptococci, staphylococci, etc. With the invasion of the infecting organism there is an increase of the blood-supply and an infiltration with polymorphonuclear cells; the increased blood-supply tends to dilute and wash out the excess antiferment, and the ferments liberated from the pus-

cells, with that already present, overcome the antiferment remaining, and softening occurs.

The sudden discharge of the softened caseous material into the bronchi as a result of the action of the iodin would also explain the occurrence of hemorrhages, as it would leave unsupported any blood-vessels traversing the mass whose walls had been involved in the tuberculous process to a degree that would render them unable to withstand the blood-pressure without some outside support. We must bear in mind the possibility and, in fact, probability, that with the softening and absorption of these caseous areas, the tubercle bacilli may be disseminated. The action of the iodids in causing a general reduction in the antiferments of the serum must also be remembered; this reduction in the serum antitrypsin would aid in bringing about the softening and removal of the caseous material.

SYPHILIS

Clinical experience teaches us that in the tertiary stage of syphilis iodin is almost a specific in bringing about the amelioration of symptoms and the disappearance of lesions, and yet little is known concerning the means by which these results are obtained. In reviewing the literature it was found that literally hundreds of articles had been written on the action of iodin in syphilis, but not one gave a rational explanation.

In order to arrive at some understanding concerning the action of iodin in syphilis two points must be considered: First, Does it destroy the infecting organism? Or, second, does it merely bring about resolution of the process, and leave the infecting organism to produce similar lesions?

Authorities on this subject are unanimous in the belief that iodids are not curative in the sense that they prevent the return of the lesions; this can be accomplished only when the iodids are combined with some other form of treatment. Tomasczawski[32] found that the administration of iodin does not prevent the development of experimental syphilis in monkeys and rabbits; neither has it a curative action; but iodized animals recovered quickly when given mercury. That iodin does penetrate into syphilitic tissues has been shown by O. Loeb.[33] This author made quantitative determinations of the amount of iodin in the blood and lymph-glands of syphilitic patients who had received large doses of sodium iodid. He found that the unsoftened lymph-glands contained 3.3 times and the softened glands 6.2 times the

32. Tomasczawski: Deutsch. med. Wchnschr., 1910, xxxvi, 653.
33. Loeb, O.: Arch. f. exper. Path. u. Pharmakol., 1912, lix, 108.

amount of iodin present in the blood. He states that most of the iodin was present as an organic compound which was insoluble in alcohol.

Wells criticizes the technic used by Loeb, and states that he always obtained larger amounts of iodin in the blood. In reply to the criticism of Wells and Hedenberg, Loeb[34] states that iodin is not present in syphilitic tissues merely as the result of diffusion, but that it exists as a true chemical combination with the constituents of the tissues.

As experimental work and clinical observations have demonstrated that the iodids do not destroy the infecting organism, we must assume that the results obtained are due to the power the iodids possess of causing resolution of the lesions present. That this actually occurs will be attested to by every clinician of experience.

Owing to its scarcity we have not been able to examine material from gummas, and so we are unable to state positively that the anti-enzymes present are similar to those found in tuberculous caseous matter; but it is probable that the same agents are active here as in those anemic infarcts in which autolysis does not occur. If this assumption is correct, it is not difficult to explain why large gummas rapidly disappear when the patient is brought under the influence of iodids. It is due to the fact that the unsaturated fatty acid radicals which inhibit autolysis have become saturated with iodin. As soon as this occurs, the ferments which are present, or which may be brought in, become active, autolysis takes place and the necrotic tissue is absorbed. Here, also, the local action of the ferments is made less difficult by the reduction of the antienzyme in the circulating blood. It must be borne in mind that the iodids are not remarkably effective in the earlier stages of syphilis when necrosis of tissue is not so evident.

If the foregoing interpretation of the action of iodin is correct, it gives the clinician a rational idea of what he is accomplishing when he gives iodids to a patient in the tertiary stage of syphilis. According to this view, iodin neutralizes the action of the agents which prevent solution and absorption of necrotic tissue, and at the same time lays bare to the action of the real germicidal agent the infecting organism which previously had been protected by the necrotic tissue. With the exposure of the infecting organism, such agents as mercury and salvarsan would be much more effective.

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34. Loeb, O.: Therap. Monatsch., 1913, p. 178.

Case 1.—B. S. Luetic. Positive Wassermann; no lesions.

TABLE 1.—Inhibition in Case 1

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/ 9/14	0	80	79	46	19	5
5/ 6/14	34	88	79	60	0	0
5/14/14	120	75	80	19	0	0

Treatment was commenced with 10 drops of a saturated solution three times a day, and the patient ordered to increase 1 drop with each succeeding dose.

The differences are very slight, if any, when large amounts of serum are used, but with greater dilution the influence of the iodids is obvious.

Case 2.—S. S. Luetic. Perforating ulcer of palate. Improved under treatment.

TABLE 2.—Inhibition in Case 2

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/ 9/14	0	85	73	48	25	5
5/ 7/14	102	86	86	33	25	0
5/14/14	120	81	44	5	0	0

The treatment ordered was the same as in the preceding case. The patient continued taking for two weeks the same dose with which she had begun; it was then increased gradually and the day the second test was made she was taking 102 drops.

Case 3.—A. M. Luetic. Tertiary stage. No symptoms.

TABLE 3.—Inhibition in Case 3

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
3/25/14	0	90	80	63	30	17
4/20/14	88	70	56	25	17	0
5/ 6/14	102	60	30	0	0	0

The patient began with the usual amount, and increased it 1 drop each dose.

Case 4.—S. Luetic. Tertiary stage. Positive Wassermann; no symptoms.

TABLE 4.—Inhibition in Case 4

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
3/25/14	0	92	86	38	26	26
4/14/14	80	88	80	38	20	5
4/21/14	101	88	85	75	30	20
4/29/14	120	80	56	30	5	0
5/13/14	180	92	85	50	30	0

The treatment ordered was the same as in the preceding cases. At the time the last specimen of blood was taken the patient showed symptoms of iodism with well-marked rash. It is interesting to note that two cases showed iodism, and in both the antitryptic strength of the serum was as high as, if not higher, than in untreated cases.

Case 5.—N. M. Non-syphilitic.

TABLE 5.—Inhibition in Case 5

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
3/25/14	0	89	89	69	17	5
5/20/14	?	83	63	38	0	0
5/26/14	45	75	80	56	25	0

The patient took her medicine irregularly. The amount taken at the time the second test was made is not known; but the results indicate that she was taking more than when the third test was made.

Case 6.—J. P. Secondary stage. No symptoms.

TABLE 6.—Inhibition in Case 6

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
3/25/14	0	90	90	90	33	25
4/14/14	40	90	92	70	30	20
4/21/14	84	88	88	Lost	33	30
4/29/14	100	88	80	75	19	19
5/13/14	145	92	88	48	5	0

The usual treatment was given.

Case 7.—S. F. Luetic. Positive Wassermann. Psoriasis-like lesion.

TABLE 7.—Inhibition in Case 7

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/14/14	0	90	90	80	26	19
4/25/14	96	81	80	56	19	5
5/ 6/14	114	83	69	40	19	5
5/11/14	168	81	88	52	5	0
5/23/14	200	86	70	63	0	0

The treatment ordered was the same as in the preceding cases.

Case 8.—G. H. Luetic. Tertiary stage. Skin lesions on the hands.

TABLE 8.—Inhibition in Case 8

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/14/14	0	89	88	50	25	20
5/ 6/14	70	81	80	40	5	0
5/25/14	100	88	78	80	60	0

The usual treatment was given. At the time the last test was made the patient presented evidences of iodism, with a well-marked rash. Here again the antitryptic strength of the serum is increased.

Case 9.—J, McD. Luetic. Secondary stage.

TABLE 9.—Inhibition in Case 9

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
3/30/14	0	90	90	84	75	25
5/ 6/14	100	85	85	52	25	0

The same treatment was instituted.

Case 10.—J. C. Luetic. Tertiary stage. Aphasia.

TABLE 10.—Inhibition in Case 10

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
5/ 1/14	0	94	71	44	26	0
5/28/14	90	75	68	25	0	0

The patient received the same treatment.

Case 11.—J. D. Non-syphilitic.

TABLE 11.—Inhibition in Case 11

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/ 6/14	0	89	70	56	17	5
5/25/14	?	85	80	76	Lost	0
5/29/14	54	87	80	Lost	25	0

The patient did not return to the clinic until about six weeks after his first visit, and he took no medicine during the greater part of this time. Treatment was started again on his second visit.

Case 12.—T. A. Luetic. Tertiary stage; no symptoms.

TABLE 12.—Inhibition in Case 12

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/29/14	0	81	78	38	20	0
5/13/14	...	83	81	38	Lost	0
5/23/14	125	85	85	77	20	0

Treatment commenced April 29, 1914, with 10 mm. potassium iodid three times a day, and patient increased the dose at irregular intervals. May 26, 1914, patient was taking 125 mm.

Case 13.—E. W. Luetic. Secondary stage. Positive Wassermann; no symptoms.

TABLE 13.—Inhibition in Case 13

Date	KI	Serum				
		0.1 c.c. Per Cent.	0.075 c.c. Per Cent.	0.05 c.c. Per Cent.	0.025 c.c. Per Cent.	0.01 c.c. Per Cent.
4/ 6/14	0	90	88	56	28	5
4/27/14	0	87	90	69	40	5
5/16/14	126	75	52	5	0	0

The patient continued taking the same quantity two weeks, and then discontinued it altogether until May 27, when she was started on 15 drops three times a day.

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CHONDRODYSTROPHIA FŒTALIS.

NOTES ON THE PATHOLOGICAL CHANGES IN FOUR CASES.

By W. G. MacCallum, M. D.,

Department of Pathology, Columbia University, New York.

The condition is one so well known and described in detail [182] in so many recent papers that there is little excuse for any further description of cases which does not bring definite new information as to the ætiology or the mechanism of ossification. Nevertheless, the opportunity to study the organs of internal secretion and the peculiarities of the lines of ossification and the cartilage may justify what is intended as a preliminary to a further study, especially of the cartilage.

The first case was that of an old woman from whom no history of the occurrence of any similar deformity in her family could be elicited. She was moderately intelligent and could care for herself and perform ordinary housework, but was irascible and quarrelsome. No especial clinical history could be obtained, but the autopsy revealed the following:

Anatomical Diagnosis.—Achondroplasia—great obesity. Shortening of all long bones. Atrophy of epiphysis cerebri.

History.—Catherine D., aged 75 years. The body is that of a most extraordinarily deformed woman 45 inches (112.5 cm.) in length (Fig. 1). The head is enormous with nose flattened back so that the nostrils look almost straightforward. Forehead large. Cheeks fat and puffy. There are two teeth in the upper jaw and none in the lower. The body is enormously fat—must weigh 150 pounds or more. Arms are absurdly short reaching scarcely to the umbilicus. The legs are similarly short and the hands and feet short and thick.

Measurements:

Upper arm 17½ cm.
Forearm 15 cm.
Hand to tip of finger 15 cm.
Crest of ilium to external malleolus 57½ cm.
Head (occipital protuberance to glabella) 58 cm. cir.
Antero-posterior measurements 31 cm.

[182] Transverse measurement from ear to ear 31.5 cm.
Length of face 31.1 cm.
Nostrils 4 cm. wide
Circum. of thigh 60 cm.
Sternum 15 cm. in length

[183]

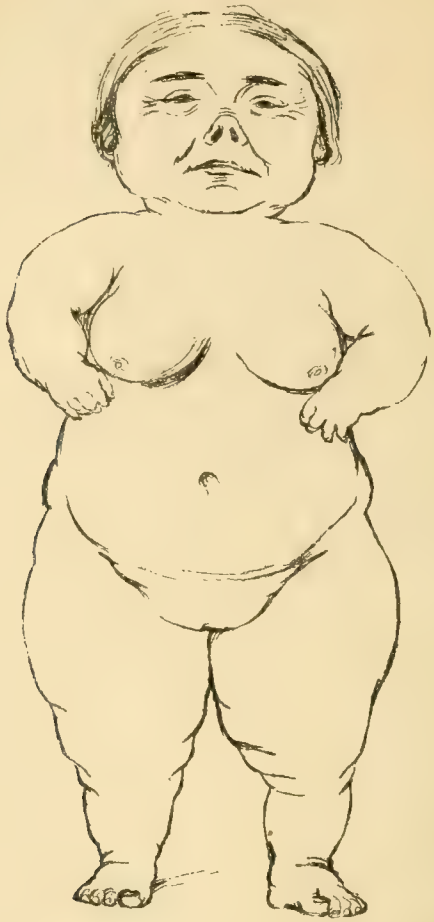


FIG. 1.—Sketch of the body of the first case of chondrodystrophia. A woman aged 75.

[182] On incision the subcutaneous fat is very abundant, 5 cm. over the abdomen. The peritoneal cavity is dry and the surfaces smooth. Omentum, mesentery, etc., enormously loaded with fat. Appendix is long and cord-like—its mesentery swollen with fat. The pelvic organs are quite free of adhesions.

The chest is very short. The costal cartilages are apparently [182]
ossified but very soft and easily crushed through. No distinct swelling of costochondral junction. Diaphragm at fourth rib on right, fifth space on left. Pleural cavities are free of fluid. No adhesions. Pericardial cavity contains no fluid. No adhesions.

Heart.—Not enlarged and very soft. Its surface is smooth and covered with fat. Relatively little blood escapes on incision. The endocardium is everywhere smooth. The mitral valve is slightly thickened and shortened—not obviously insufficient. The aortic valves are slightly thickened but competent. The other valves are delicate. The heart muscle is dark red and very soft.

Lungs.—Not very voluminous. The pleural cavities are relatively small. The bronchi are calcified in their large branches and in their main trunks. On section the lung substance is dark red, somewhat œdematous in places but otherwise air containing throughout.

Thymus.—A great mass of fat lies in the anterior mediastinum and the whole was kept for further study but no definite thymus tissue could be made out.

Thyroid.—The gland measures 4 x 3 x 1 cm. on the right, slightly less on the left. Its tissue is of normal color, soft, with little colloid and not scarred. Two *parathyroids* were found [183]
toward the lower pole to the median side of the thyroid. They measured about 9 x 5 mm., were very thin and flat and brown. The other two have not yet been found.

Carotid Glands.—Were preserved and seem not enlarged nor especially altered.

Lymph Glands.—Were not enlarged in the neck and very few were found—one or two were deeply pigmented. Nothing abnormal was seen in the tongue or pharynx.

Spleen.—Was rather large and soft. Measures 11 x 6 x 4 cm. All the elements were plainly visible.

Pancreas.—Embedded in fat. Its tissue looked rather soft and œdematous. The pancreatic duct is easily traced to the papilla.

Liver.—Weighed 2¾ lbs. (1350 gm.). The left lobe is very small. The right on section shows a soft rather shrunken liver substance with much fat content. The acini are indistinct in outline. The gall-bladder contains a large calculus which fills the whole bladder. The cystic and common ducts are normal.

Stomach and Duodenum.—Normal. Small intestine and colon not opened. Rectum normal.

Adrenals.—Are irregular in outline. Not notably altered in size. The cortex is readily torn apart so as to leave a cavity in place of the medulla. In the irregular cortex are many irregular nodules. Left measures 4.5 x 2.5 x 0.5 cm. Chromaffin nodules are preserved along the gangliated cord.

[183] *Aorta.*—Is distinctly inelastic and rather uniformly set with small patches of sclerosis throughout its whole length. It is 4 cm. in circumference in the mid-lumbar region. Other arteries, including carotids even into the cavernous sinuses, very sclerotic and dilated.

Kidneys.—The left measures 11 x 5 x 5.5 cm. Buried in fat. Capsule strips off easily and smoothly. Surface is rather swollen looking. Lobulations are normal. On section the blood-vessels are rather thick. Cortex is 7 mm. in thickness, striations straight.

Bladder.—Normal.

Uterus, Tubes and Ovaries.—The ovaries are of moderate size and very little scarred for their age. The right measures about 1.5 x 1 x 1 cm. The left is rather larger, 2 x 1.5 x 1 cm. On section there are some old remains of corpora lutea. The ovaries are not unduly atrophied. The tubes are normal. Uterus is of normal size and appearance. Measures about 7 cm. in length. There is a rather curious soft elongated mass, apparently of muscular tissue, without lumen in the situation of the left round ligament. The mucosa of the uterus is gray and moist and contains some small cysts.

Pelvis.—Is greatly contracted. The internal conjugate diameter (promontory to symph. pubes) measures only 6 cm. The transverse inner diameter is 10 cm. The birth canal will admit only three fingers in the antero-posterior direction.

The femur (preserved) is phenomenally short (22 cm.). The extremities are slightly enlarged.

Nothing abnormal could be seen in the vertebral column although the antero-posterior curves were rather marked.

Brain.—The dura is very adherent to the calvarium which is thickened somewhat. The base of the skull was not carefully examined but seemed very slightly if at all divergent from the normal. The foramen magnum was about the normal size and the spinal cord was not encroached upon. The sella turcica was very slightly enlarged, chiefly to hold the dilated and sclerotic loops of the carotid arteries. The *hypophysis* is in gross about normal in size, form and consistency.

The surface of the brain is marked by great atrophy of the convolutions and widening of the sulci which were filled with fluid. The œdematous pia was pigmented in places with yellow granular pigment.

On section the cerebral ventricles were found markedly dilated with clear fluid. The third ventricle is widened and a commissure extends across it as a free cord. The *epiphysis* or pineal gland is reduced to a minute almost transparent fragment of tissue upon its recognizable stalk. It does not seem to be one-twentieth as large as it should be. Spinal cord not removed.

Microscopically, the organs showed little if any abnormality. [183]
Hypophysis, parathyroids, adrenals, pancreas, carotid glands and ovaries seemed perfectly normal. The pineal gland as stated was very small, not more than 2 mm. in diameter, but so far as it is preserved its structure is normal. It seems probable that the decrease in size may represent a senile change. The thyroid shows microscopically only such changes as are very commonly seen in the thyroid of old people, namely, a slight scarring or increase in the density of the fibrous framework. The epithelial cells are desquamated in some alveoli and colloid is not abundant, but on the whole it seems functionally competent. The femur and a rib were studied microscopically but except for the rarefaction of the cancellous bone, also incident to age, no abnormality could be observed. Naturally, the epiphyseal line was no longer to be made out and the articular cartilage appeared normal.

Nothing could be learned then from the conditions of the organs in chondrodystrophy at such an advanced age to throw any light on the nature of the disease, except that all of the organs of internal secretion seem normal and that the remaining organs are also normal. Evidently nothing is left after the cessation of growth in the bones to explain the perversion of that growth.

The other three cases were in infants, two of which were found in the museum of the College of Physicians and Surgeons, while the third was a premature child at about the [184]
seventh month, born of apparently healthy parents. The autopsy on this case was performed by Dr. A. M. Pappenheimer who gave me the material.

The two infants found in the museum had been preserved in alcohol for many years and no history was available. The internal organs were softened and useless for microscopical or even gross study, so that nothing can be said of their organs of internal secretion. The base of the skull and the long bones could be investigated, however. In the third case, born of healthy parents, the thyroid was quite normal, except for a slight desquamation of the epithelial cells. The parathyroids were normal. The thymus was of normal size, its medullary portions rather indistinctly marked out against the cortex. The adrenals, pancreas, spleen, liver, etc., showed no abnormalities. Unfortunately the hypophysis and pineal gland were lost at the autopsy and could not be studied.

In all three there was extreme shortening of the arms and legs. The head was relatively large but the deformity or

[184] retraction of the nose so commonly met with was not particularly striking. Vertical sagittal slices were cut through the base of the skull in each infant and cleared up by the method of Spalteholz. These showed that in every case the two portions of the sphenoid bone and the basilar portion of the occipital bone were widely separated by cartilage, so that in these cases, at least, the premature synostosis described by Virchow and held accountable for the retraction of the nose has not occurred. Kaufmann points out that it occurs in only a part of the cases. Bones were excised from each case for study. The femur was in every instance extremely short, the incurved shaft appearing extremely small in contrast to the large masses of epiphyseal cartilage, which overhang it almost like a cap on all sides (Fig. 2). The cartilaginous trochanter in each instance seemed disproportionally large, so that the head of the femur appeared as a small projection beneath it.

On cutting through the femur longitudinally the shaft was found to be very dense and hard and the periosteum thick. The cartilage, in which of course no centers of ossification had as yet appeared, was not particularly softened, although microscopical examination revealed a change in the structure somewhat resembling that described by Kaufmann and by Marchand and Kirchberg as chondromalacia.

This was true of the first and third cases which were so much alike that they may be described together, while the second case presented a different appearance.

In the first and third of the infants longitudinal microscopical sections through the femur and tibia showed the following: The periosteum of the shaft forms a thick layer under which a dense bony wall is deposited. Within this cortex the cancellous bone which makes up the shaft is extremely compact and dense even though many of the lamellæ contain some central remnants of cartilage. The periosteum is prolonged past the end of the ossified material and turns inward as a spur into the substance of the cartilage, although it does not extend far. This evidently represents the periosteal lamina described by Urtel, Kaufmann and many others as forming a barrier against the endochondral ossification. Much dispute has taken place as to whether this were merely an inclusion of the peri-

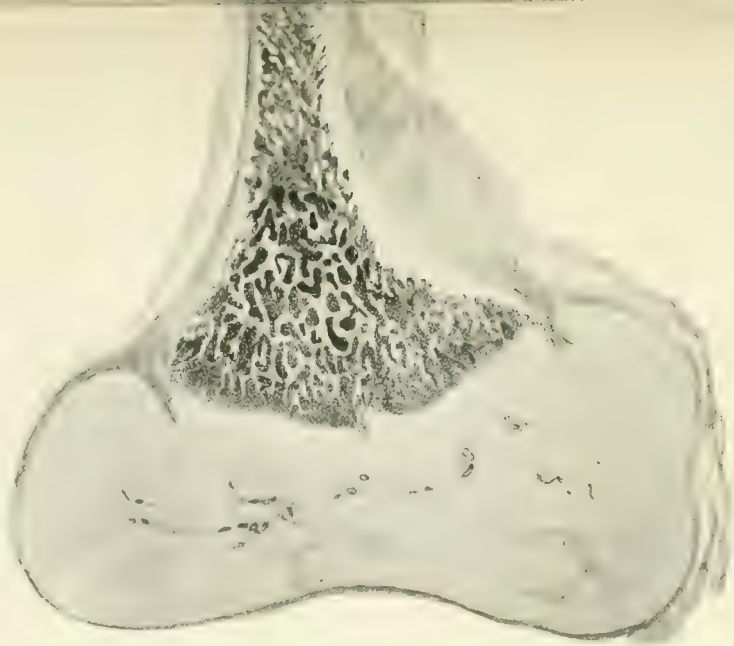


FIG. 2.—Section of femur of infant, Case 2, showing abnormal arrangement of cartilage and periosteal spurs.

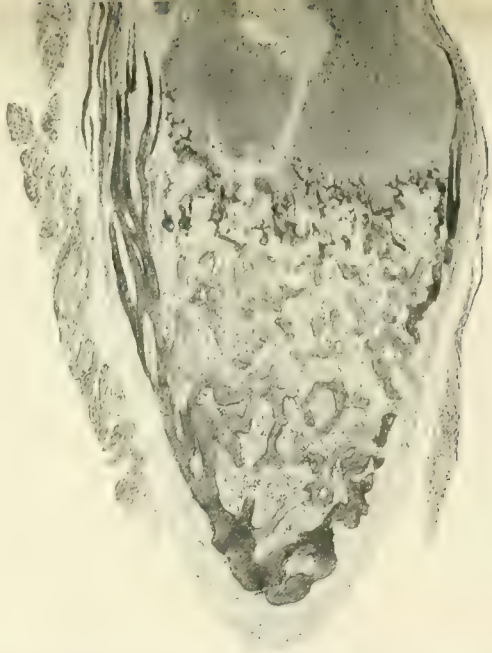


FIG. 3.—Costochondral junction, Case 2, showing irregularity of the line of ossification and extensive calcification of cartilage.

osteum or an active ingrowth. In these cases it extends so [184]
short a distance that in itself it could hardly interfere much with ossification—instead it appears to be merely the end of the periosteal sheath of the shaft over which the exuberant cartilage has poured down the outside like overflowing paraffin which solidifies.

Study of the cartilage shows that in all the distal parts, and especially perhaps in that which extends backward outside the periosteum, there is a peculiar disappearance of the normal homogeneous matrix, so that the cartilage cells are single or in little groups which hang together in a network. In the meshes there are numerous fibrils and many branching cells surrounded by fibrillæ. Indeed, in places, and especially about the numerous blood-vessels, the cartilage is transformed into a felt-like connective tissue, throughout which groups of cartilage cells are scattered. This is very different from the distal parts of the normal epiphyseal cartilage in which the elongated spindle-shaped cartilage cells lie in every direction, but closely embedded in the homogeneous matrix. It will be seen from the drawing that this overflowing of the cartilage removes much of it from a position in which its cells might eventually be presented for invasion by the marrow spaces and, indeed, leaves the end of the shaft of the bone covered by a relatively thin layer of cartilage. Probably much of what seems an excessive production of cartilage is, through this softness and shifting from the range of the ossification process, rendered useless for the further growth of the bone.

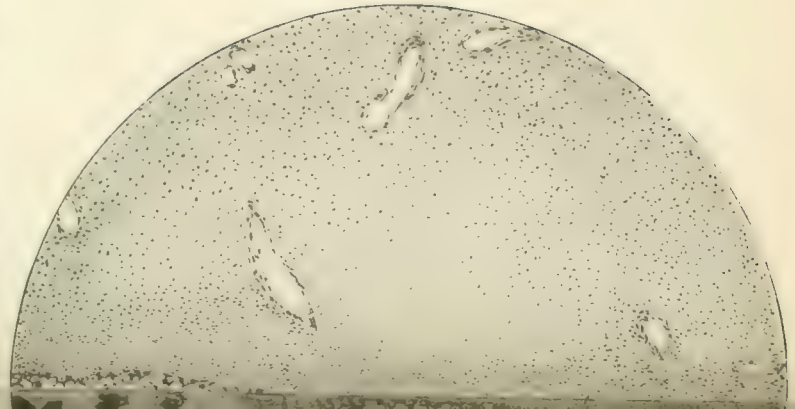
In the remainder, as the time of ossification is approached, a preparatory zone becomes recognizable, in which the cartilage cells are flattened and arranged in columns which run vertically to the line. Although in the normal this perfectly regular phalanx is maintained in spite of the swelling of the cartilage cells, it is rather different in these cases. The columns lose much of their regularity and the invading marrow spaces with their blood-vessels become correspondingly irregular, so that the line of ossification, while in general fairly even, is vague and indefinite. The ossification process obviously continues but varies in intensity at different points. In some sections, and especially in those through the costochondral junc-

[184] tion, the line of ossification is exceedingly irregular (Figs. 3 and 4).

Not only is the order of the cells destroyed but the process itself seems somewhat modified. Quite extensive calcification of the cartilage matrix is found between the unaltered cartilage cells, and ragged branching masses of this calcified material, often including cartilage cells, are found to extend quite down among the lamellæ of the cancellous bone. These gradually become ossified by the application of osteoblasts, but imprison for a long time the cartilage cells which were included in their central parts.

Thus, if somewhat slow and irregular, ossification does still go on and produces dense cancellous bone. From the section, however, it is impossible to say how much cartilage is actually presented for ossification in this way and it seems that it is
[185] on that that the extreme retardation in the longitudinal growth of the bone depends.

The second infant was very badly preserved and the details can hardly be made out in sections of the bones. Nevertheless, it can be seen that the process is rather different from that in the other two. The cartilage at the ends of the excessively short bones is abundant but does not pour over the ends of the bone shaft in the way described for the others. Microscopically, it is not found to differ markedly from the normal, being composed of a complete and homogeneous matrix in which the cells are embedded (Fig. 5); but the preparatory zone presents no columns of cells. The cells are all flattened it is true, but they form no columns even quite down to the ossification line nor swell up as they do in the normal; consequently, the line of demarcation between cartilage and bone is perfectly even and is composed on the one side of flattened cartilage cells which present an unbroken front to the marrow cavity. At the very margin there are some which have become calcified with the matrix into a layer which is continuous at places with masses or laminæ of what appears to be calcified cartilage from a previous epoch when ossification was proceeding more actively. Lower still these fuse with dense bony laminæ. Here the endochondral ossification seems to have been at a standstill at the time of the child's death and prob-



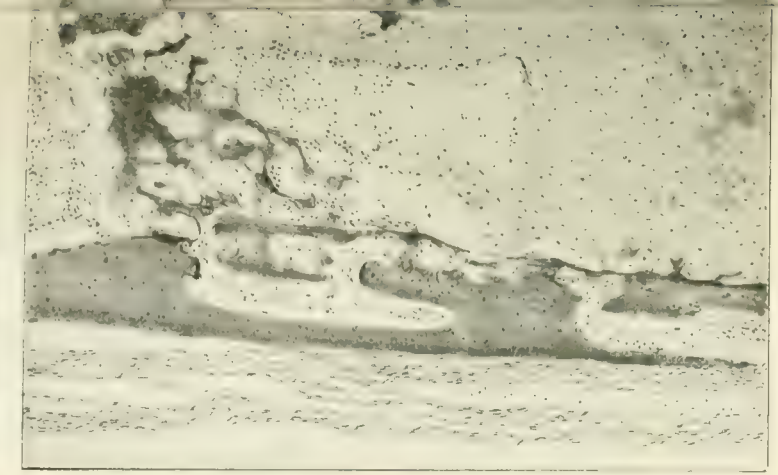


FIG. 4. A portion of the same more highly magnified, showing lack of column formation in the cartilage. Periosteal bone formation extends some distance past the line of ossification.

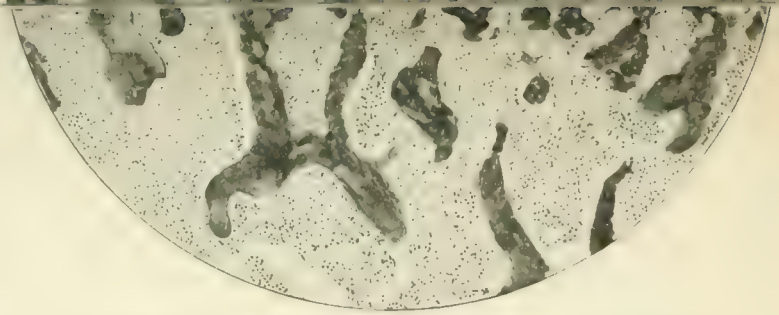


FIG. 5. Line of ossification of femur in Case 3, showing a lack of column formation in the cartilage and consequent lack of ossification.

ably this accounts well for the extreme shortness of the bones. [185]
The shaft of the femur was less than 1 cm. long, although the child was at full term or thereabouts.

These alterations in the process of ossification, even at their severest, seem far less calculated to check the growth in length of the bone than such destructive changes along the line of ossification as are seen in congenital syphilis. Even in infantile scurvy, or in rickets, the disturbances of endochondral ossification seem more severe than those in chondrodystrophy, and yet they have relatively little effect in shortening the bones; while the chondrodystrophic, if he survives, is a dwarf. The explanation probably lies in the fact that although the line of ossification is somewhat irregular, ossification is not really in abeyance. It proceeds with all vigor on the part of all the other osteoblasts and in the case of the line of ossification; the fault lies undoubtedly with the cartilage which fails to present its cells in orderly fashion and rapidly enough to produce a bone of normal length or to keep pace with the periosteal growth. Why this is so is not yet clear but it is enough to justify Kaufmann's term Chondrodystrophia fœtalis.

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THE CHOLESTEROL METABOLISM OF THE HEN'S EGG DURING INCUBATION.

By J. HOWARD MUELLER.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

(Received for publication, February 20, 1915.)

The large amount of work which has been carried out dealing with the various aspects of the physiology and pathology of cholesterol, leaves many points to be cleared up before an accurate understanding of this complex question is possible. It has long been known that cholesterol occurs in the blood and tissues in two principal conditions; namely, free, and combined with the higher fatty acids as cholesterol oleate, palmitate, and stearate.[1] Little or nothing is known concerning the importance of the relative proportions of these two forms. Until the elaboration by Windaus[2] in 1910 of a quantitative method which made possible the determination of the two fractions separately, it was impossible to obtain any accurate information on the point. Since that time many interesting investigations have been carried out, principally upon the cholesterol of blood in diverse physiological and pathological conditions, by Gardner and coworkers,[3] Henes,[4] Havers,[5] and others.

There is as yet, however, no clear conception of the origin of the esters of cholesterol in the body, nor of their possible reconversion into free cholesterol. As a result of the work of Gardner and others, it is now pretty definitely established that

[1] K. Hürthle: *Ztschr. f. physiol. Chem.*, xxi, pp. 331–359, 1895.

[2] A. Windaus: *ibid.*, lxv, pp. 110–117, 1910.

[3] C. Dorée and J. A. Gardner: *Proc. Roy. Soc., Series B*, lxxx, pp. 212–239, 1908; lxxxi, pp. 109–128, 1909. M. T. Fraser and J. A. Gardner: *ibid.*, lxxxi, pp. 230–247, 1909; lxxxii, pp. 559–568, 1909–10. J. A. Gardner and P. E. Lander: *ibid.*, lxxxvii, pp. 229–236, 1913–14.

[4] E. Henes: *Deutsch. Arch. f. klin. Med.*, cxi, pp. 122–145, 1913.

[5] K. Havers: *ibid.*, cxv, pp. 267–289, 1914.

all the cholesterol of the body is taken in with the food, and that there is no synthesis of cholesterol in the body. Under normal dietary conditions the cholesterol of the food must be quite largely in the uncombined condition. It may be said that there are two sources of origin for it; namely, vegetable food and animal food. Since there is no synthesis by animals, the original substance must be one of the so called "phytosterols,"[6] substances which are probably of the same or nearly the same chemical formula as cholesterol itself, and which appear to be readily changed over by animals into true cholesterol. As far as I am aware, these phytosterols occur in plants only in the free or uncombined form. Hence the esters occurring in the animal body must be products of its own metabolism.

It seemed possible that a study of the cholesterol content of the egg during the period of incubation might throw some light on the relation of cholesterol and cholesterol esters in the life processes of animals, and that here, from the nature of the case, conditions would be ideal for studying this relation. The total cholesterol of eggs in varying stages of incubation has been investigated by Mendel and Leavenworth[7] and by Ellis and Gardner[8] in order to determine whether there might be an increase attributable to a synthesis. Their results were negative in that respect, their figures showing, however, a small but constant decrease when averages of a number of determinations have been made. This they attribute to experimental error, but it may be said in passing that there is some evidence[9] in support of the belief that bile acids are formed from cholesterol. Should this prove to be the case a slight decrease might be satisfactorily accounted for.

As regards the cholesterol ester content of chick embryos, Hanes[10] observed microscopically the presence of doubly re-

[6] A. Windaus and A. Hauth: *Ber. d. deutsch. chem. Gesellsch.*, xxxix, pp. 4378–4384, 1906. A. Windaus and A. Welsch: *ibid.*, xlii, pp. 612 616, 1909.

[7] L. B. Mendel and C. S. Leavenworth: *Am. Jour. Physiol.*, xxi, pp. 77–84, 1908.

[8] G. W. Ellis and J. A. Gardner: *Proc. Roy. Soc., Series B*, lxxxi, pp. 129–132, 1909.

[9] J. Lifschütz: *Ztschr. f. physiol. Chem.*, xci, pp. 309–328, 1914.

[10] F. M. Hanes: *Jour. Exper. Med.*, xvi, pp. 512–526, 1912.

fractile fat droplets in the liver from the fifteenth day up to hatching, and also their persistence for about two weeks or more in the chick after hatching; but he notes their absence in the liver of the adult chicken. These doubly refractile droplets, becoming isotropic at about 40° to return again to the anisotropic condition upon cooling, are recognized as consisting for the most part of cholesterol esters. As far as I know this is the only record of such an observation, and since Hanes made no chemical analyses, it seemed desirable to do so. Hanes related the appearance of the esters to the process of calcification, and this question will be taken up more in detail after a consideration of the analytical results obtained.

Method of analysis.

The eggs used were all from the same breed of chicken. Beginning with the third day, an egg was broken every second day, the embryo cut into small pieces with scissors and placed together with the yolk sac and other contents, in alcohol. After twenty-four hours the alcohol was changed and later was replaced by ether. Following one change of ether, the solid material was dried and ground in a mortar. The combined alcoholic and ether extracts were evaporated to dryness and taken up in ether, the ether-insoluble part being rubbed up with dry sodium sulphate and added to the other solid material. This was now extracted for forty-eight hours in a Soxhlet apparatus, the extract transferred to a volumetric flask and made up to 100 cc. with ether. 10 cc. of this were used for the determination by the Windaus method[11] with a modification very similar to that of Fraser and Gardner.[12] The ether was evaporated off, about 30 cc. of alcohol were added, heated to boiling on the water bath and a 1 per cent solution of digitonin in 90 per cent alcohol was added. The alcohol was then evaporated off in a current of cold air from an electric fan, and the drying finished either by gentle warming, or by placing in a vacuum desiccator over sulphuric acid for a few hours if necessary. It was then taken up in ether, filtered through a tared filter paper, using as the tare a similar filter paper, which during the analyses was put through the same washing as the one containing the precipitate. This consists in washing thoroughly with ether, drying, and washing with boiling water. The two papers are finally dried at 105° to 110° for an hour and weighed. The ether washings are evaporated, the residue saponified with 2 gm. KOH in alcoholic solution for two hours on a water bath, then diluted with water and extracted with ether, using five 25 cc. portions. The com-

[11] Windaus: *loc cit.*

[12] Fraser and Gardner: *loc. cit.*

bined ether extracts are shaken out with two small portions of distilled water to remove small quantities of alkali which always contaminate the extract. The ether is then evaporated and the determination of cholesterol made as before. This final determination gives the cholesterol present originally as the ester.

The following protocol shows the results obtained.

DAY OF INCUBATION	FREE CHOLESTEROL	COMBINED CHOLESTEROL	FREE CHOLESTEROL IN TOTAL
	gm.	*gm.*	*per cent*
3.	0.2159	0.0242	89.92
5.	0.2470	0.0252	90.74
7.	0.2643	0.0396	86.97
9.	0.2985	0.0354	89.40
11.	0.2249	0.0243	90.25
13.	0.1993	0.0389	83.66
15.	0.2035	0.0827	71.10
17.	0.2115	0.0732	74.29
19.	0.1789	0.0831	68.28
21.	0.1575	0.1109	58.68
*2.	0.1539	0.1026	60.00
*9.	0.1289	0.0469	73.32

*Age of chick

It is a recognized weakness of the Windaus method that in the presence of any considerable quantity of other lipoid material, values for the combined or ester fraction are too high. Since this condition obtains in these analyses, it may probably be safely assumed that the 10 to 12 per cent of ester found during the first two weeks is more than is really present, and that most of the cholesterol present is free.

While only one analysis was made in each case, the general trend of the process is very evident, consisting in little or no change up to about the end of the second week, and from then until the twenty-first day a rather sudden increase in the amount of combined cholesterol, causing a lowering of the proportion of the free to a minimum of about 60 per cent of the total. Following hatching of the chick there appears to be a slow decrease in the proportion of esters, but more analyses are necessary for definite conclusions as to this.

In general the results obtained bear out to a surprising degree the conclusions drawn by Hanes from microscopical observations. It should be noted, however, that by no means all of the esters

present are contained in the liver, nor is the cholesterol of the liver all in the combined form. The livers of five twenty day embryos were analyzed together, yielding 0.0179 gram of free cholesterol and 0.0516 gram of combined cholesterol, while in the combined bodies of the five embryos, with the yolk sac, still partially unabsorbed, there must have been about 0.3 gram of combined cholesterol.

As to the significance of this rather sudden production of cholesterol esters during the final week of incubation, it was the idea of Hanes that they were connected with the process of calcification in the following way. Plimmer and Scott[13] showed by analyses of the phosphorus compounds of the incubated egg, that, corresponding to the period of calcification, *viz.*, from the fourteenth day on, there was a sudden drop in the amount of lecithin or "phosphorized fats" which had previously remained pretty constant. Since the decrease in the vitellin phosphorus was gradual and fairly constant during the whole three weeks of incubation, it seemed plain that the phosphorus necessary for calcification came from a breaking down of the lecithin. Hanes believes that in this breaking down, which he suggests may take place in the liver, some of the liberated fatty acids, which might otherwise exert a toxic action, are combined with the cholesterol to form non-toxic substances, the cholesterol esters.

This theory fits the known facts well. It is at once evident, however, that approximately 0.10 gram of cholesterol is inadequate to combine with the fatty acids liberated in the breaking down of 0.7 to 0.9 gram of lecithin. Still it may be readily believed that the greater part of the acid could be oxidized or recombined as a neutral fat with glycerol, only a small part being left unprovided for, to be taken care of by the cholesterol.

As to the ultimate fate of these accumulations of esters, little can be said with assurance. It is certain that there is a decrease in the proportion of esters, beginning soon after hatching. Röhmann,[14] Schultz,[15] Cytronberg,[16] and Gardner and Lander[17] have

[13] R. H. A. Plimmer and F. H. Scott: *Jour. Physiol.*, xxxviii, pp. 247–253, 1909.

[14] F. Röhmann: *Berl. klin. Wchnschr.*, xlii, pp. 1993–1994, 1912.

[15] J. H. Schultz: *Biochem. Ztschr.*, xlii, pp. 255–261, 1912.

[16] S. Cytronberg: *ibid.*, xlv, pp. 281–283, 1912.

[17] Gardner and Lander: *Biochem. Jour.*, vii, pp. 576–595, 1913.

reported the splitting of cholesterol esters on autolysis of liver, or mixtures of liver and blood from various animals, presumably by the action of some specific enzyme. Rothschild[18] believes that he has microscopical evidence for the splitting of these esters in the Kupffer cells of the liver. We have been unable to obtain results suggesting enzyme action in the liver either of chicks just hatching, or of adult chickens, but propose to investigate this point further.

There seems, then, to be evidence that cholesterol is, at least in part, a protective substance, having for its function the power to combine with and "detoxify" the fatty acids, substances which when introduced into the circulation exert a marked toxic action through hemolysis.

It is worthy of note in this connection that cholesterol can also unite with the various saponins, substances which are also hemolytic. Upon this union the Windaus method depends for estimation of cholesterol, the compound with digitonin (a saponin) being of definite composition, and very difficultly soluble in alcohol, from which it crystallizes.

CONCLUSIONS.

1. The cholesterol of the newly laid hen's egg is practically all in the free condition.

2. During the period of incubation this condition obtains until about the thirteenth day, from which time there is a gradual esterification until, at the time of hatching, over 40 per cent of the cholesterol present is in the form of esters.

3. The esterifying cholesterol may function as a detoxifying substance, with which the toxic fatty acids, set free from lecithin during the latter stage of embryonic development, combine to form harmless esters.

[18] M. A. Rothschild: *Proc. N. Y. Path. Soc.*, xiv, pp. 159–171, 1914.

[Reprinted from Proceedings of the New York Pathological Society.
N.S., Vol. XV, No. 3, March, 1915.]

A CASE OF MULTIPLE MYELOMA

W. G. MACCALLUM, M.D.

Clinical History.—B. C., a woman aged 53, was admitted to the Presbyterian Hospital December 5, 1915, complaining of stiffness of neck and shoulders. This appeared gradually during the past three months but was not associated with any pain. On admission neck was held very stiffly, apparently entirely on account of spinal rigidity, as the muscles were not tense. There was a deep groove in the ribs running parallel with the sternum just outside the costo-sternal junction. The ribs were easily moved or bent and on very slight pressure gave the impression of being crushed. Thoracic and abdominal organs showed nothing abnormal. The spine was held rigid throughout. Both femora and tibiæ showed outward and forward bowing. No tenderness anywhere.

Blood count, examination.—

Leucocytes	9,000
Polymorphonuclears	54 per cent.
Lymphocytes	41 per cent.
Hæmoglobin	80 per cent.

On December 18 she developed signs of pneumonia and died five days later. The urine showed a moderate amount of albumen with hyaline casts but no Bence Jones protein.

X-ray photographs showed atrophy of the cervical vertebræ with destruction and collapse of the third, fourth and fifth. Areas of rarefication were thus demonstrated in many of the other bones including especially the ribs and long bones.

At the *autopsy* there was found a widespread confluent lobular pneumonia. The alterations of especial interest concerned the bones.

The ribs were rarefied and softened to such a degree that in

most places they could be crushed easily between the fingers. They were especially thinned and greatly depressed on each side of the sternum and in these broad grooves nearly every one showed several fractures, often marked out by the growths of callus tissue.

The eleventh dorsal vertebra had collapsed to a certain degree, so that there was a slight angular deformity at that point. Three of the cervical vertebral centra had collapsed in the same way, bending the neck forward into a position in which it had become somewhat rigid.

One humerus was cut open and its cortex proved to be not more than 1 mm. in thickness, while the large narrow cavity was devoid of any cancellous lamellæ and filled with a mottled grayish red opaque marrow.

If we may judge from the X-ray photographs the same rarefication had occurred in the other humerus and in the bones of both forearms, as well as the femora tibiæ and fibulæ. No fractures had occurred in those bones and there was no sign of a tumor growth at any place. Many diagnoses were ventured to explain the rarefication of the bones and the many fractures but even at the autopsy the mere cellular character of the marrow exposed in the humerus did not suggest a tumor formation. The cut surfaces of the vertebræ were perfectly homogeneous and except for a slight opacity, not different from normal bones.

MICROSCOPIC FINDINGS

Smears from the marrow stained by Giemsa and Hastings' methods showed a remarkable uniformity of cells. Nearly all were rounded or oval cells of moderate size with rather deeply stained nucleus and practically no granules in the blue stained cytoplasms. There was found a pale halo around the nucleus. Occasional suggestions of basophilic granulations in small numbers were found.

Sections reveal a marrow composed in places of the normal cells in their usual arrangement, but nearly everywhere there were found compact masses of cells of one type pushing aside the normal marrow and extending everywhere into contact with the

bone. Many fragments of cancellous lamellæ were found isolated in such masses. This tissue which was composed of cells corresponding with those found to be predominant in smears did not as a rule impinge directly upon the bone but seemed always separated from it by a thin line of cells flattened almost like endothelial cells, which may have been osteoblasts. Nevertheless the effect in causing the destruction and wasting of the bone was extraordinary.

The cells which make up this tumor tissue were not like the myeloblasts described as forming the tissue in a case with numerous myelomatous tumors published in the *Journal of Experimental Medicine* some years ago (Vol. 6) but resembled more closely plasma cells. Whether they correspond exactly with plasma cells it is difficult to say, but after observing recently two cases in the laboratories of Dr. Norris and Dr. Warren, in which the whole character of the growth was very similar to this except in that definite tumors were found, it seems that two distinct types of myeloma must be recognized. Of these one is composed of myeloblasts, the other of cells practically identical with plasma cells. The explanation of the absence of albumoses in the urine is not clear but there have been recorded several other cases in which the urine was free from these bodies.

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AORTIC ANEURYSM PERFORATING SUPERIOR VENA CAVA

W. G. MACCALLUM, M.D.

Clinical History.—H. G., woman, age 33, was admitted to hospital fifteen times between January, 1913, and her last admission, in the summer of 1914.

She had led a dissipated life with much abuse of alcohol and tobacco and very possibly had had syphilis. She entered the hospital complaining of shortness of breath and swelling of heart, face and neck. Ten days before admission in January, 1913, patient felt that her face was swollen; the same night she woke up with a choking sensation accompanied by pain. The neck was much swollen and the face bloated.

The physical examination showed œdema of the upper part of the chest and great dilatation of the capillaries in that region. A tracheal tug was

felt. There were diastolic and systolic murmurs over the heart and a definite Corrigan pulse. The veins in the epigastric and hypochondriac regions were dilated and tortuous. Eye grounds normal.

During the next month the cyanosis and subcutaneous œdema in the upper part of the chest increased. There was a good deal of cough with rusty sputum.

In February of 1914 these conditions had become accentuated, marked dyspnœa and continuous murmur heard over the back as low as the fifth dorsal vertebra. There was a large accumulation of fluid in the right chest.

In May the superficial veins over the upper part of the chest were very prominent. Wassermann reaction positive.

June 16, 1914, admitted for the last time with still more exaggerated symptoms of the same character.

Death occurred on August 26.

At the *autopsy* the left lung was found to be a good deal compressed and to lie against the vertebral column. The heart was removed, together with the large vessels and the lungs and upon dissection it shows the following:

The heart itself is large, its walls somewhat thickened; the pulmonary artery is pushed forward by a saccular enlargement of the aorta beneath. This sac measures 5 to 6 cm. in diameter in each direction, so that the pulmonary artery is not really much compressed. The superior vena cava is delicate until it reaches the region of the auricle, where it becomes thickened and somewhat rigid and is adherent to the aortic dilatation. The thoracic portion of the aorta shows no dilatation or intimal change up to the end of the ascending arch. The large vessels of the arch pass off normally. They, like the aorta, are delicate and elastic.

On opening the heart and cutting through the aortic ring and the dilated aorta it is found that the left auricle is normal; the left ventricle dilated; mitral valves normal; its trabeculæ and papillary muscles flattened. Just beneath the aortic valves there are linear rough elevations which look like a beginning reaction to aortic insufficiency. The aortic valves themselves are fairly competent by the water test; their edges, however, are thickened and roll into a cord, so that insufficiency must have been possible, with dilatation, and probably existed all the time. Aortic orifice measures 7 cm. in circumference. The tricuspid and pulmonary valves are normal. Right ventricle is rather large; its walls

measure 7 mm. in thickness; left ventricular wall measures 19 mm. in thickness. Coronary orifices are normal. Just above the coronary orifice there is a sudden dilatation of the aorta which ends as suddenly with the precipitous edge at the upper end of the ascending part of the arch. It is nowhere roughened or covered by thrombi, but the inner surface is very irregular and there are numerous small sacculations as if new small aneurysms started out from the wall of the old one. One of these, about 12 mm. in diameter, arises from the lower posterior part of the main aneurysmal sac and extends over toward the superior vena cava and presses against it; indeed, it practically pierces a way through the middle of the vein and divides it into two channels, fusing with the further wall of the vein, so that it looks like a bridge across the cavity of the vena cava, when it is exposed by opening that vessel. The blood was forced to pass on each side of this bridge and the two channels must have been still further narrowed by the thickening of the vein wall at this level. They now measure about 10–15 mm. in diameter. Under such circumstances there was no connection between the aortic lumen and that of the vein, since the aneurysmal saccule merely pushes its way through the middle of the vein to the opposite side; nevertheless it undoubtedly caused a good deal of obstruction to the return of blood from the head.

But the aneurysmal sac is found to have burst laterally as it passed across the vein, and a probe from the main aneurysm into the saccule emerges into the vein through a hole which proves to be in the wall of the bridge turned uppermost. This aperture is surrounded by a thrombus on the venous side and it was obviously through this that the blood rushed.

One receives the impression that this actual perforation may have taken place rather recently but the symptoms which it could have produced, such as murmurs and cyanosis, were already present to such a degree on account of obstruction of the vein and the valvular murmurs that it is surprising that the diagnosis could have been reached so accurately as it was.

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SOME NEW SPECIES OF ECTOPARASITIC TREMATODES

By G. A. MacCallum, M.D.
Department of Pathology,
COLUMBIA UNIVERSITY, NEW YORK.

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FIGURES

Fig. 132. *Diplectanum teuthis.*

Fig. 133. *Diplectanum lactophrys.*

Fig. 134. Comparative view of cirri of several species of *Diplectana*:—

a. *Diplectanum balistes.*
b. *Diplectanum teuthis.*
c. *Diplectanum longiphallus.*
d. *Diplectanum aequans*—after Norman MacLaren.
e. *Diplectanum lactophrys.*

Fig. 135. *Diplectanum balistes.*

Fig. 136. *Diplectanum longiphallus.*

Fig. 137. *Atalostrophion sardae.*

Fig. 138. The entire intestinal tract of young *A. sardae.*

ABBREVIATIONS

See Plates

b. ej.—bulbus ejaculatorius
c.—cirrus
d.—disc
d. e.—ductus ejaculatorius
ex. d.—excretory duct
ex. p.—excretory pore
es.—esophagus
g. a.—genital atrium
g. p.—genital pore
i.—intestine
m.—mouth
m. b.—muscular bands
oot.—ootype
o. s.—oral sucker
ov.—ovary
ovd.—oviduct
ph.—pharynx
p. p.—pars. prostatica
pr.—prostate
p. s.—penis shell
s. g.—shell gland
s. p.—sensory papillae
s. r.—seminal reservoir
t.—testis
u. g.—unicellular glands
ut.—uterus
va.—vagina
v. d.—vas deferens
v. s.—vesicula seminalis
vit.—vitellaria
v. r.—vitelline reservoir

SOME NEW SPECIES
OF
ECTOPARASITIC TREMATODES

By G. A. MacCallum, M.D.
Department of Pathology,
COLUMBIA UNIVERSITY, NEW YORK.

Introduction.

The Director of the New York Aquarium has been good enough during the past two or three years to place at my disposal numerous exotic and other fishes which have died there. I greatly appreciate the privilege, for it gives me the opportunity to secure biological specimens which I could not obtain otherwise.*

Among these there have been found many forms of parasites of new species and even genera, which it has been thought should be published, and, with that object in view, in the following paper five new species and one new genus are submitted. The form from *Sarda sarda, Atalostrophion* is a particularly interesting trematode.

When one considers that these worms are always parasitic and that in many instances the death of their host depends upon the numbers which cause the infestation, it becomes interesting to know how they are propagated. This fact also makes the study of their anatomy more interesting since almost all of them are hermaphroditic. They are supplied with two complete sets of generative organs and when these are thoroughly described there is but little left to say about the worm's anatomy, since they form the main portion of its body. Although their habits

*Dr. MacCallum has kindly autopsied and reported upon the cause of death of large numbers of fishes from the Aquarium during the past few years, only a few of which are mentioned in this paper. The importance of such studies in fisheries work is very great, and an aquarium offers special advantages for this work on account of the greater opportunity for infection and the protection afforded diseased fishes, which, in natural conditions, would be quickly destroyed by their enemies. This paper is properly a contribution from the Biological Laboratory of the New York Aquarium.—*C. H. T., Director of the Aquarium.*

are interesting, where they may be studied, it is not often that an opportunity is afforded to see them alive and pursuing their usual mode of life. This, however, is quite possible in some cases, for instance, where Microcotyle infest the gills of certain fish. By exposing the gills they may be seen literally covered with the worms hanging on the gill filaments while they suck the blood from under the delicate mucous membrane. On the gills, too, may be seen the eggs deposited in hundreds and held in position by the tangled filaments which are attached to either end of the egg. As soon as they are hatched they are prepared to fasten themselves on the gills and to take up the reproduction of their kind and to continue the work of their ancestors. The great irritation caused by all this action induces an outflow of thick mucus, covering the gills and preventing the access of the water to their surfaces, so that the fish even may be suffocated. In addition to this they cause great depletion of blood which soon renders the fish exsanguine and causes its death.

Another strange habit with most of these parasitic worms is, that they have a particular locality or habitat where they confine themselves in or on the body. Thus the *Microcotylidae, Diplectana* and *Octocotylidae* are found no where else than on the gills, and the former worm is at least the cause of death of ninety per cent of the angel and butterfly fishes in the tanks of the Aquarium. They are probably not so plentiful, and consequently not so fatal to these fish in their natural habitat, the open sea.

DIPLECTANUM

Sub-Family Gyrodactylidae

Genus *TETRAONCHUS*

Sub-genus *Diplectanum*

Among the ectoparasitic trematodes there are few which while being as small, are yet more interesting than these forms.

They have been classed in the order *Heterocotylea* and included by Van Beneden and Hesse in the family of *Gyrodactylidae*. As far as known now, they are confined to the gills of marine fishes and we have no record of them until 1857, when Wagener was fortunate enough to discover three members of the

family. These he named *Diplectanum aequans,* found on the gills of *Labrax lupus; Diplectanum pedatum* on the gills of an unknown fish of the Julis family, and *Diplectanum echeneis* on the gills of *Sargus rondeletii.* However, other than naming them he gave little or no description of the worms by which they might be recognized.

In 1862 Van Beneden and Hesse did the genus more justice, but it was not until 1904 that it was much more completely described by MacLaren, who has endeavored thus to make *D. aequans* the type species.

In 1862 Van Beneden and Hesse described the worm discovered by them as *D. sciaena* from the gills of *Sciaena aquilla,* one of the drumfish.

Parona and Perugia discovered and described a form which they called *D. aculeatum.* We have then thus far five members of this sub-genus described as follows:

> *Diplectanum aequans,* Wagener, 1857, Diesing, 1858, MacLaren, 1903; *Diplectanum pedatum* Wagener 1857; *Diplectanum echeneis,* Wagener, 1857; *Diplectanum sciaena,* Beneden and Hesse, 1863 and 1864; *Diplectanum aculeatum,* Parona and Perugia, 1890.

In 1904, when Norman MacLaren wrote very fully upon *D. aequans,* he also summed upon what had been written of the other forms to that date.

Diplectanum teuthis, nov. sp.*

(Fig. 132)

On May 22, 1914, I found *Diplectanum teuthis* on the gills of a *Teuthis hepatus,* a small worm of this species which differs in many particulars from any of those hitherto described, consequently I have ventured to name it *D. teuthis* and to call it a new species. It is only .75 mm. in length by .10 mm. in width. However, its anatomy is fairly distinct so that a reliable description may be given of it.

*All of the forms described in this paper were found in material from the New York Aquarium.

The body of the worm is elongated, slender and ends posteriorly in a trumpet-shaped disc, which is armed with four strong yellow chitinous hooks of a peculiar shape. There are also in this locality a number of stiff hair-like processes which extend some distance anteriorly from the disc along the posterior end of the body. I wish to draw attention to the shape of these hooks, which are shown in Fig. 134. The hooks in the disc have as their foundation of attachment, two blocks of chitinous material. These are placed, one anteriorly and the other posteriorly in the wall of the hollow disc. They act with a certain amount of motion as if jointed, and thus allow free action of the hooks, which are under the control of the worm.

The head is narrow and unarmed with a notch at the anterior end which marks the situation of the mouth, and on each side of this there are three tactile papillae which stain more highly than the surrounding structure. These areas seem also to furnish some secretion, since small ducts may be seen proceeding backward from them as far as the pharynx. Their function is, as in all of these worms, to afford mucus or saliva to the pharynx and esophagus and to enable them to feel their way about. Anterior to the pharynx are four ocular spots. The pharynx is large relatively and is succeeded by a very short posterior pharyngeal esophagus, since it divides almost at once into the intestinal ceca. On each side of the pharynx and beginning of the intestinal ceca are a number of unicellular glands. A short distance posterior to this angle but also in the center of the body is the genital pore surmounted by a very prominent organ, the chitinous cirrus, which in this species is of the shape of a sickle with the handle.

The *male genital* apparatus consists of a single testis, oval in shape and relatively large. It is situated posterior to the ovary and the vas deferens passes dorsally over the ovary on its way to the large ductus ejaculatorius; this duct gives off a tube which proceeds somewhat backward to enter the bulbus ejaculatorius, being surrounded just before entering that organ by the prostatic gland. The bulbus ejaculatorius is a more or less round organ or muscular bag through which the vas deferens passes to the base of the cirrus. The cirrus is peculiar. Its shape being that of a sickle, including the handle, which latter

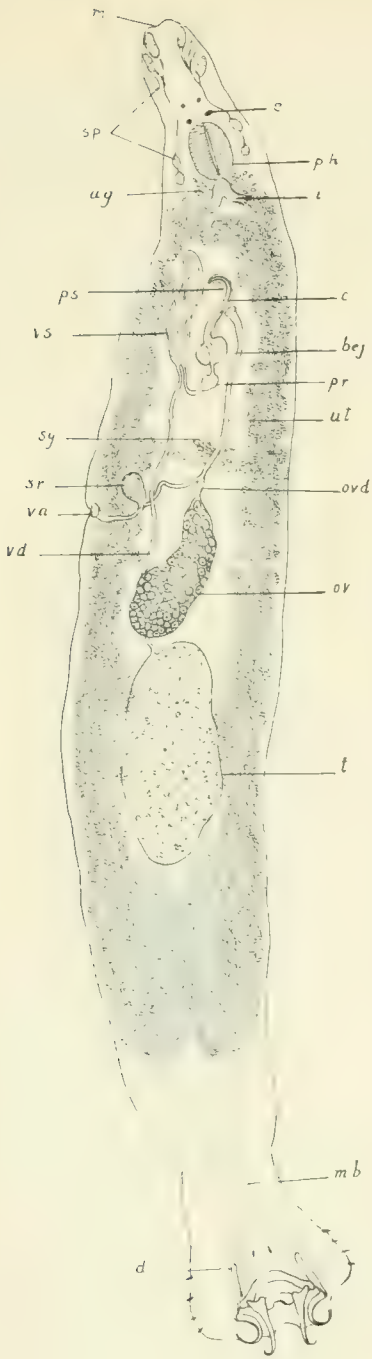

FIG. 132. *DIPLECTANUM TEUTHIS*

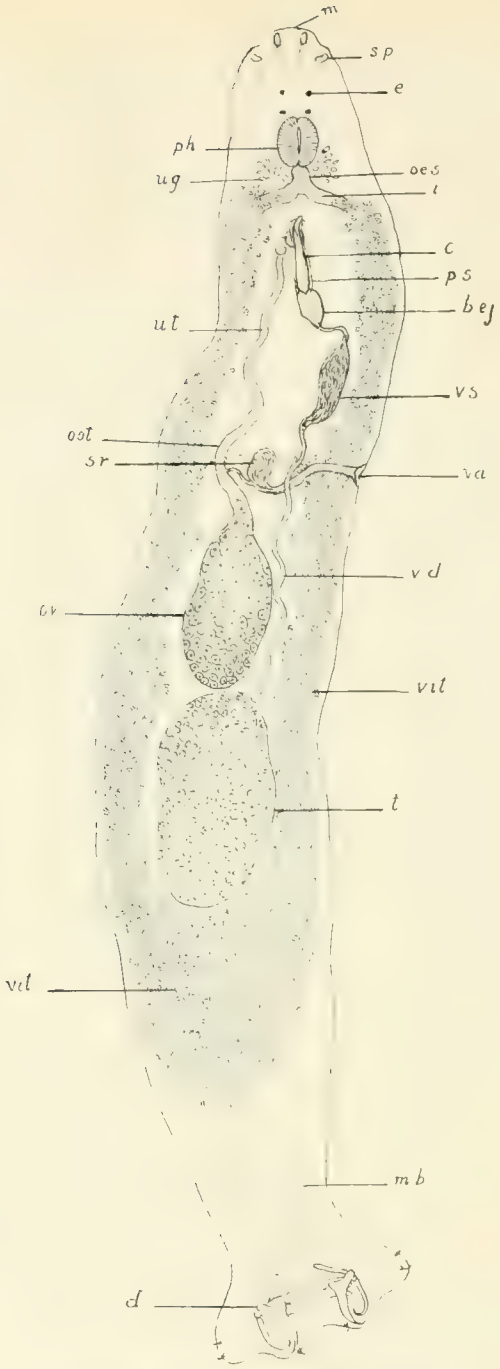

FIG. 133. *DIPLECTANUM LACTOPHRYS*

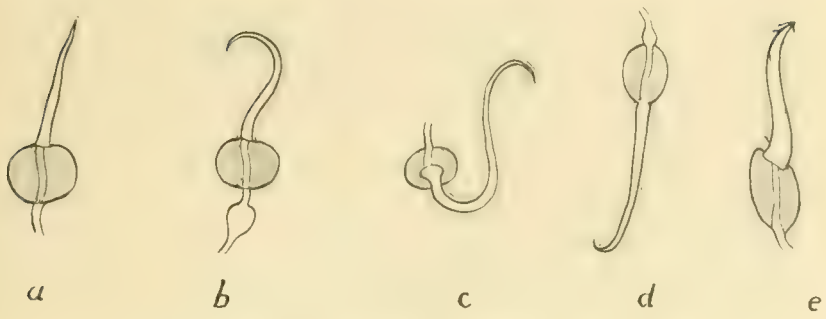

FIG. 134. CIRRI OF SEVERAL SPECIES OF *DIPLECTANA*

seems to form part of the ejaculatory apparatus. The organ itself is a hollow tube, much bent and composed of yellow chitinous material (Fig. 134b).

The *female genital* apparatus consists of a reniform ovary situated about the middle of the body. The oviduct is short and the genital junction is a short distance anterior to the ovary. On the right side of the worm may be seen the opening of a vagina which joins the seminal reservoir near the genital junction.

The vitellaria are plentiful and extend on each side of the body near the pharynx to a short distance anterior to the disc; small vitelline ducts are sent almost horizontally to the genital junction from each side.

The uterus extends from the ootype near the genital junction to an atrium near the base of and to one side of the cirrus.

Measurements of *Diplectanum teuthis*:

Length	.75 mm.
Width	.10 mm.
Length of cirrus	.04 mm.
Diameter of disc	.08 mm.

Diplectanum lactophrys, nov. sp.

(Fig. 133)

On February 19, 1915, there were found on the gills of a cow fish, (*Lactophrys tricornis*), a colony of small *Diplectana*

which owing to a few characteristics not possessed by other members of this family, must necessarily make it constitute a new species. It is small, being only 1 mm. in length by .10 mm. in width; it has, however, the usual general conformation of the genus.

Its body is elongated and narrow and of almost an equal width throughout, except at the posterior end where the hook disc is somewhat wider. The disc is, however, quite different from any of the forms hitherto described; it is more footlike in shape,—in fact, in some specimens it is quite the shape of a foot, but ordinarily it extends equally on each side of the end of the body. This shape is caused largely by there being on each side a prolongation of skin, which is armed with two small hooks. Then on each side of the notch in the centre of the distal margin there is fixed another small hook. In the centre of the disc towards its margin are two small blocks of yellow chitinous tissue, which give attachment to the bases of the main hooks of the disc, of which there are, as usual, four—two attached to the block placed in the anterior wall of the disc and two attached to the posterior block.

These blocks are of a peculiar shape and differ in each species (Fig. 133). The blocks are each separated into parts which like joints allow of the motion of the hooks at the will of the worm. As will be seen also if observed closely, the hooks are not of the same shape as those of other members of the genus. In the posterior region may be seen the muscular bands which extend from the disc up into the body and which control the movements of the disc with its hooks. Some bands, if not the chief ones, are seen to pass from one side of the disc to the opposite side of the body. This is probably for the more varied adjustment of the hook disc.

The head in its general conformation and the organs of the body generally are much the same as in the description given of the type except in the cases of the cirrus which is quite different and will constitute one decided point in the diagnosis of the species.

The *male genitalia* are the single large roundish testicle, which lies posterior to the ovary in the center of the body. It gives off from its anterior part the vas deferens which runs

along the left side of the abdomen to empty into a rather large irregularly shaped vesicular seminalis situated opposite the base of the cirrus. This reservoir furnishes a small tube which enters the bulbus ejaculatorius and passes to the pointed base of the cirrus which points to and sometimes overlies the genital pore. The cirrus here is long and straight except near the tip where it becomes curved and pointed. At its base it is provided with an oval bulbus ejaculatorius quite elongated while in nearly all of the other species this is round or nearly so. The base, too, of the cirrus is pointed where the tube or vas deferens joins it; it then shortly widens until it suddenly narrows to the portion outside of the bulbus ejaculatorius. The cirrus proper, however, is of firm chitinous tissue which cannot alter in shape even during conjugation, so that as it is quite prominent it shows a good index of the species (Fig 134e). The cirrus is enclosed in a sort of shell or outer cover and in this instance the penis seems to be armed near the tip with fine spicules, and there appear a few of these spicules also near the base. It is barely possible that this condition may be owing to faulty refraction, still as almost all of the specimens show the same it probably is true.

The *female genitalia*, as usual, consist of an ovary, oval in shape, becoming pointed anteriorly and from which part arises the oviduct—this is not long before it receives the duct from the seminal reservoir and the vitelline ducts; the ootype follows and merges into the uterus, being surrounded here by the shell gland. The uterus terminates with a somewhat flaring mouth at the genital atrium. The vagina opens at the left side of the body when it is looked at from the ventral side of the body. The *viteliaria* are plentiful, extending on both sides of the body from near the pharynx to near the posterior end and across the body posterior to the testis.

These worms are all ectoparasitic, living on the gills of marine fishes. They are not as a rule found in great numbers, although there may be many more than appears since they cannot be seen in situ on the gills, and they do not wash off readily on account of the firm hold of the hooks. The fish as a rule which are infested with them become thin and flabby, and finally die, probably owing largely to the ravages of the worm when in great numbers.

Measurements of *Diplectanum lactophrys*, n. sp.

Length of body	1 mm.
Width of body	.10 mm.
Diameter of disc	.15 mm.
Length of cirrus	.10 mm.

Diplectanum balistes, nov. sp.

(Fig. 135)

On January 13, 1913, on the gills of a trigger fish, (*Balistes carolinensis*) were found a few examples of a small worm which on close study proved to be a *Diplectanum* and a new species, inasmuch as it differs from any others of the genus examined. It is but .55 mm. in length by .09 mm. in width. The body is elongated and, unlike many of the other members of the family, it becomes decidedly and abruptly smaller near the discal end and the disc itself is very different from that of the others. Instead of being trumpet-shaped it is merely a straight tube armed with four hooks and about six smaller ones. The large ones measure .02 mm. and the smaller ones .003 mm. The hooks also are different in shape from those of the other forms described (Fig. 135).

The *male genitalia* consist of one testis situated near the middle of the body and just posterior to the ovary. The vas deferens runs dorsal to the ovary in a convoluted form to join a fusiform vesicula seminalis which lies to the left of and slightly posterior to the genital pore. At its anterior end it narrows into a small tube which enters the bulbus ejaculatorius and passes through to enter the cirrus, which in this instance is an almost perfectly straight tube (Fig. 134a) and in the specimen under observation lies pointing anteriorly directly across the genital pore. It is almost .04 mm. in length.

The *female genitalia* consist of the ovary lying, when viewed ventrally, partly on the testis. It is oval and narrows anteriorly into the oviduct, which is almost immediately joined by the duct from the seminal reservoir which receives the vagina from the right side. The genital junction is just anterior to the ovary and the uterus, which is short, terminates at the edge of the genital pore.

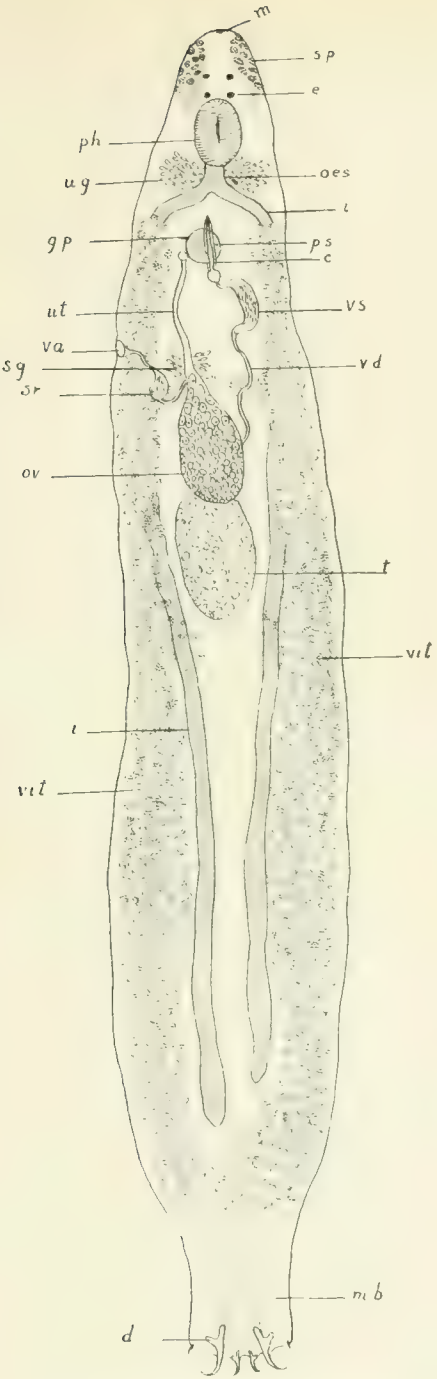

FIG. 135 *DIPLECTANUM BALISTES*

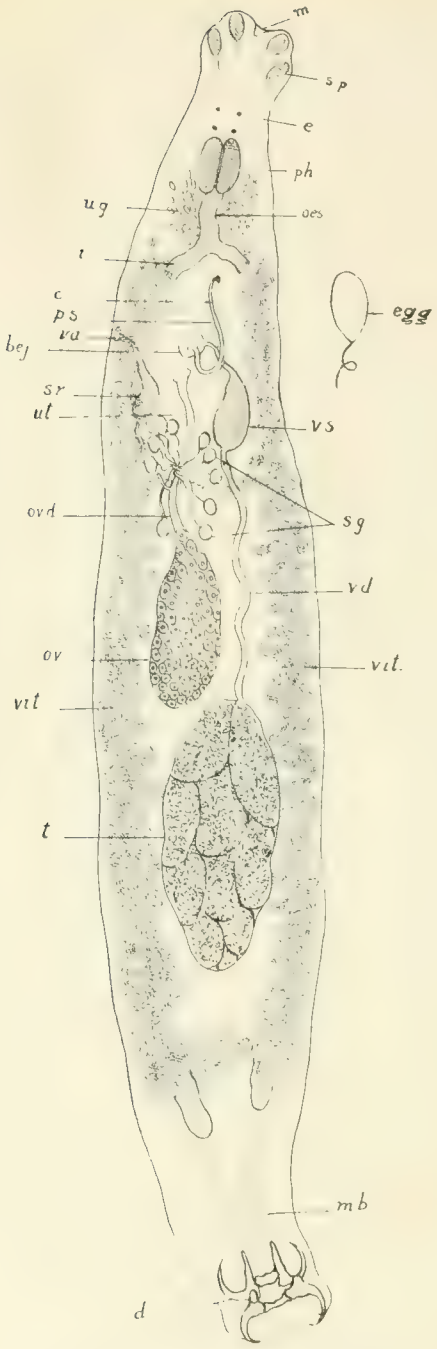

FIG. 136. *DIPLECTANUM LONGIPHALLUS*

The head is formed much like that of others of the family, a slight notch at the extreme tip and on each side a row of papillae extending back almost to the pharynx, which is large. The post-pharyngeal esophagus is short and the angle on each side of the pharynx and esophagus is filled with unicellular mucous glands. Anterior to the pharynx are the usual four ocular spots. The vitellaria are plentiful and extend almost from opposite the genital pore to the point posteriorly where the body suddenly narrows to form the disc. The hooks on the disc have their bases attached to the usual two chitinous blocks of yellow material. These are not of the same shape as in *D. teuthis* or *D. longiphallus.*

For the purpose of description, the characteristics of the worm are, that it is small, has a straight tubular disc end, four large hooks and several small ones clustered in the end of the disc, and the cirrus straight and pointed.

On account of these peculiarities the worm must be considered a new species and the name *D. balistes* is proposed.

Measurements of *Diplectanum balistes.*

Length	.55 mm.
Width	.09 mm.
Diameter of disc	.035 mm.
Width of head	.02 mm.
Length of cirrus	.04 mm.

Diplectanum longiphallus, nov. sp.

(Fig. 136)

On January 23, 1915, there were found on the gills of a spade fish, *Chaetodipterus faber*, a colony of *Diplectana,* which apparently are different from those described heretofore.

In general appearance they are like those found on the surgeon fish, (*Teuthis hepatus*) and in *Balistes Carolinensis,* or trigger fish, but in detail the anatomy is somewhat different. Like them it is small; being only about 1 mm. long by .15 mm. wide. The clinging disc seems in general form the same in almost all, a firm terminal mass with four relatively large, strong hooks attached to odd shaped chitinous blocks, which serve to support the

hooks and increase the firmness of their grasp on the tissues to which they cling. The head is similar, with the usual tactile or sensory papillae on each side, in this instance only two but somewhat larger than in the other forms. An esophagus can be made out between the pharynx and the mouth end and in front of the pharynx are the usual four ocular spots. The pharynx is large and the post-pharyngeal esophagus is longer before the division into ceca than the pre-pharyngeal portion. The genital pore is in the angle between the ceca in the centre of the body and this is surmounted by an oddly shaped chitinous cirrus, quite different from those of the forms above mentioned (Fig. 134c). In this instance it is very long and much bent or curved.

The *male genitalia* are very similar to that usual in the species but with some peculiarities. For instance the testis is single, very large and enclosed in a membranous sac, is divided into large lobes, and is more or less pointed anteriorly. It is situated posteriorly to the ovary and from the anterior end gives off the vas deferens, which passes forward to empty into a large seminal vesicle, placed alongside of the genital aperture on the left side; from this the vas passes into the bulbus ejaculatorius before terminating in the cirrus.

The *female genitalia* consist as usual of the ovary, which is large, oval and pointed anteriorly where it gives off the oviduct. The vagina is not very definite in most of the specimens before me but in most of them the situation of the seminal reservoir is seen and a tube leading to the oviduct is evident. The shell gland in this species is peculiar since it is composed of ten or fifteen large, round, cellular masses that communicate with the ootype by means of long tubes, and give the glands the appearance of being set up on stalks. The vitellaria are very plentiful and the vitelline ducts meet and join the oviduct anterior to the entrance of the duct from the seminal reservoir.

The uterus is not long and terminates with a somewhat flaring mouth near the genital pore near the bulbous end of the cirrus. Several eggs are seen in the eight or nine mounted specimens before me; one in each. These are yellow, oval and with a filament at the posterior pole,—length .02 mm. (Fig. 136.)

For the purpose of classification this form shows the following special characteristics: Shell gland unusually prominent

and composed of ten or fifteen large round glands with very long ducts. Cirrus quite long, a good deal curved and with an oval bulbus ejaculatorius which appears quite muscular. Testis lobulated. These differences from the other species described force me to regard this as a new species for which the name of *Diplectanum longiphallus* is proposed.

Measurements of *Diplectanum longiphallus*.

Length	.95 mm.
Width	.15 mm.
Length of cirrus	.25 mm.
Length of disc	.08 mm.
Width of head	.05 mm.
Egg	.02 mm.

Atalostrophion sardae, nov. gen. & nov. sp.

(Figs. 137 and 138)

During the summer of 1912, I first found on the gills of the bonito, (*Sarda sarda*) by washing, a number of fragments of a small, flat, ribbonlike worm. It was always found to be broken at the end, but when stained and mounted it was seen to contain internal organs of a tubular form; that is, there were two, sometimes three tubes containing eggs, which were portions of the uterus. Between these uterine tubes was a brownish tube containing brownish yellow granules—the vitellarium—and besides these could also be seen the tubular testis and a vas deferens containing spermatozoa. No matter how many pieces were examined from the gills, they were always imperfect; both ends being torn across. This was the case during 1912 and 1913, although very few bonitos came to autopsy in the laboratory. However, it was determined in 1914, that an attempt should be made to find out the origin of the pieces and to secure a whole worm if possible. As a result of this determination sixteen bonitos were examined in all and seven of them found to be infested with the worm. It was then discovered that the worm had its habitat not in the gills of the fish, but was found in great numbers under the mucous membrane of the branchial cavity on each side of the isthmus, and also was

found embedded in great numbers in the thyroid glands and throughout the substance of the isthmus itself.

When the muscular layers were carefully parted, the worm was seen flattened in tangled layers. Some of these were seen dangling from the surface in loops, the examinations being made under water, as this was the simplest way to secure a view of the parasites in situ. I have no doubt now that the loops are broken off by the action of the gills and water and become entangled in the meshes of the gills, doubtless for the purpose of distribution in one way, since every fragment is loaded with eggs. It is certain, however, that the local habitat of the worm is in the cellular tissue under the skin and mucous membranes of the branchial cavity and neighboring tissues of the throat, including the space between the muscular fasciculi of the muscular tissues of the isthmus.

It may be said, that a great deal of time has been spent with most careful and assiduous research in the endeavor to find a whole worm. Out of the hundreds traced and teased out only two whole worms have been secured and both of these were immature. Three or four ends, however, have been secured,—heads and tails of adults which are shown in Figs. 137 and 138. It has been concluded that it is next to impossible to tease out an adult worm from the fact that they all seem to be imperfect. One may lift carefully the mucous membrane or a layer of muscular tissue from a tangled mass which certainly has never been disturbed or injured before, and examine the specimens as carefully as possible in situ, and it is almost impossible to see a perfect end; let alone a whole worm. In consequence one must conclude that it is natural for the worm to disseminate its eggs by throwing off the parted sections, as well as by the natural exit from the uterus. The one young specimen secured is 50 mm.—nearly two inches long—and the other more developed one is about 70 mm. in length. Even though dissecting with the greatest care and using a stereoscopic lens, on no occasion have I been able to secure a piece of the body of greater length than 75 mm. This is not to be wondered at when it is known that the worm is only about .60 of a mm. wide; not more than as thick as tissue paper. Its structure, too, seems to be very delicate; breaking at the slightest touch, almost. The skin is smooth and unarmed. It is difficult to state the length of an

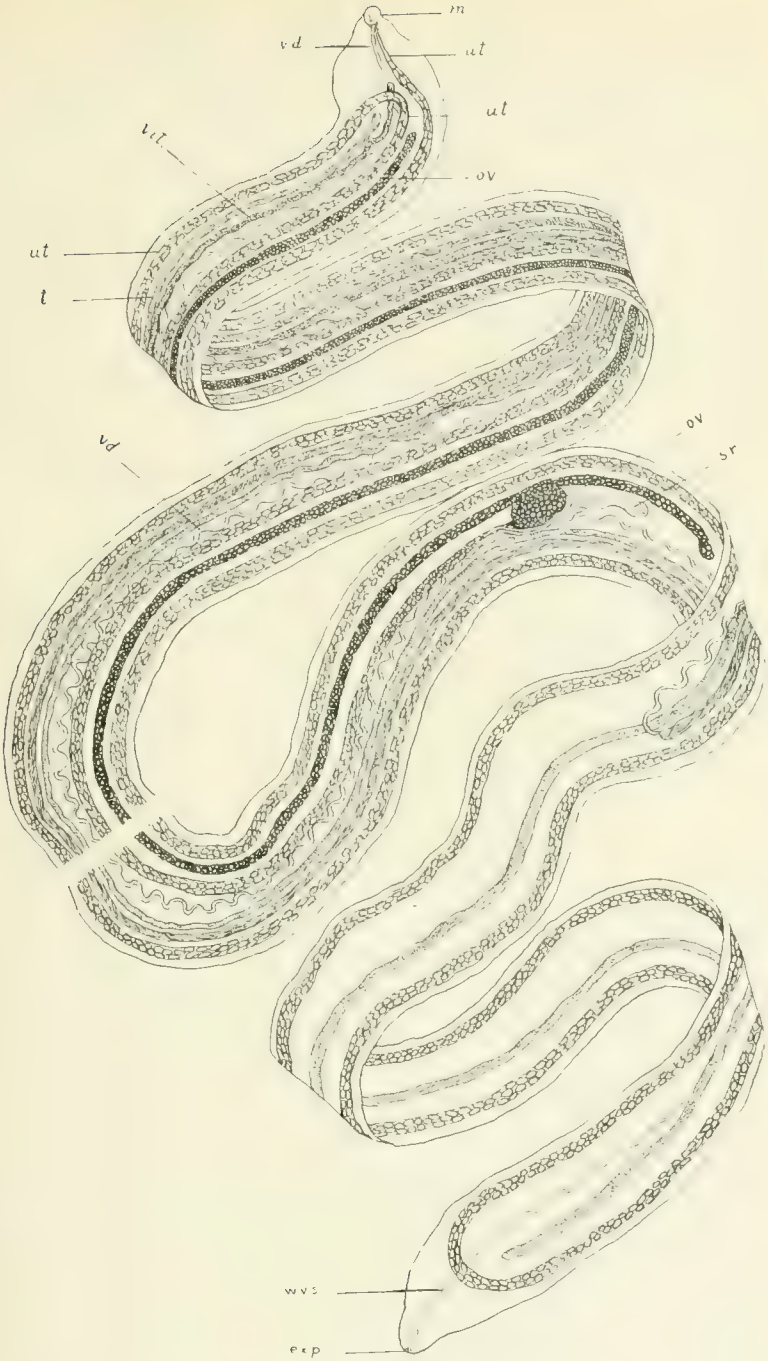

FIG. 137. *ATALOSTROPHION SARDAE*

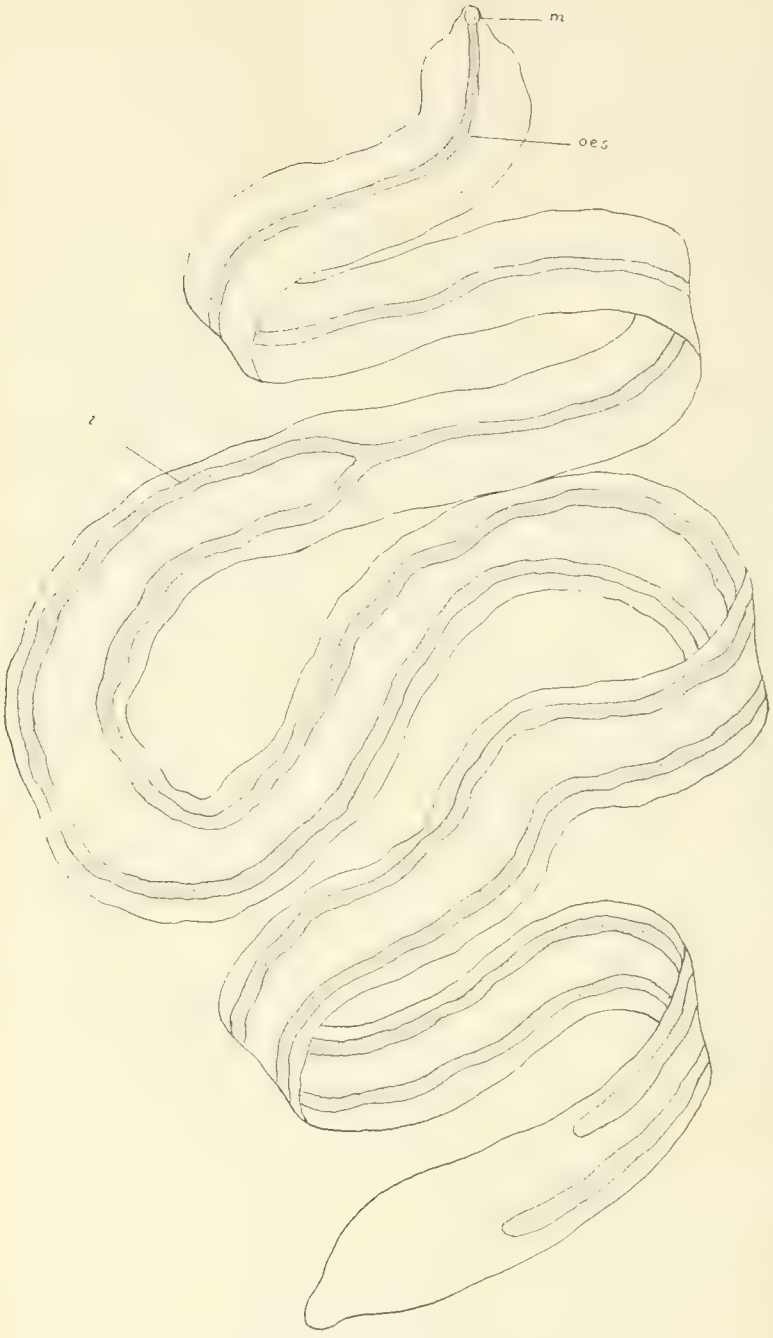

FIG. 138. INTESTINAL TRACT OF *A. SARDAE*

adult worm. I have pieces three inches long and from my experience in trying to get a whole worm by putting the pieces together, I would say that I believe five or six inches the maximum length. In the figures the worm is shown cut across, because a whole adult worm has not yet been dissected out to make a certainty about length.

The worm sometimes seems to have all the organs, and the uterus full of eggs, etc.; the form of body will then abruptly change to a much narrower and thinner shape and to a milky white color, with only a water vascular canal in the middle, running through the middle the entire remaining length, perhaps for an inch or so, and coming to a fine point or be broken off when not larger in diameter than a hair.

In one of the young worms the esophagus and intestinal ceca are plainly shown and are depicted in Fig. 138. As may be seen in the figure, the esophagus extends from the mouth about one-third of the length of the worm, when it divides into two ceca which extend nearly to the posterior end of the worm. No pharynx is seen.

The mouth is an oval opening distinctly marked out. It is slightly sub-terminal and may be observed when the worm is on its back. The anterior ends of the uterus and vas deferens plainly pass under the posterior edge or lip, and disappear in the mouth, which must therefore be the outlet for their contents. Eggs are seen in utero, a very short distance from the end, but no mouth of the uterus can be seen. The tissues of the body in this locality, as well as elsewhere outside of the organ, appear to be a fine network of muscular fibers with small cells and fine granular matter sparingly distributed. In some of the specimens the head which is very small appears to be connected with the body by a decided neck, but of course this varies as the result of muscular action. No nervous system can be made out, but a water vascular system is present and the small vessels gradually coalesce towards the tail until the main vessel often can be seen making a zig zag course to the outlet.

The *female genitalia*, as far as can be made out, consist of the ovary which is a thick somewhat curled tube extending from near the anterior end to about the junction of the posterior with the anterior two-thirds of the worm. It stains deep red with carmine. In some specimens there is an enlargement a

short distance from its posterior end in which the ova seem riper than elsewhere. Just behind this portion of the ovary may be seen a rather large seminal reservoir from which a tube passes around the ovary to join the oviduct just anterior to the ovary. Anterior to the ovary and duct from the seminal reservoir, a small vitelline duct is seen to join the oviduct, after which there is formed the ootype, which merges into the shorter division of the uterus. The eggs in this first portion of the uterus are immature and it is not until they have proceeded to the anterior end of the worm, thence to the tail and return to the head that they seem fully developed and ready to be laid, or discharged through the genital pore. They are small—.005 mm., oval yellow, without filaments and very numerous.

The vitellarium, also in tubular form, extends almost the whole length of the worm; it is somewhat nodular and stains with carmine a yellowish brown. No vagina has been seen nor has a shell gland been found.

The *male genitalia* consist of a tubular testis which extends about two-thirds the length of the worm beginning near the anterior bend of the uterus. It stains pinkish. At the posterior end of the testis the vas deferens is given off; this is as usual a convoluted tube which extends anteriorly to the atrium situated, as viewed from the ventral surface of the worm, behind the mouth. No cirrus is visible.

The characters of the worm for the purposes of classification may be given as follows: Flat, ribbonlike, very narrow throughout, but very attenuated towards the anterior end; uterus in two or three tubes extending uncoiled throughout nearly from one end to the other; the ovary also tubular, but not nearly so long as the uterus. Vitellarium and vas deferens are also single tubes; the former nearly half as long as the uterus. The testis is also tubular and the vas deferens extends through nearly three-fourths of the length of the worm to the end at the genital atrium at the mouth. Living in great numbers, tangled together under the mucosa of the gill cavity of marine fishes, *Sarda*, etc.

This worm was found in material at the U. S. Bureau of Fisheries laboratory, at Wood's Hole, and the work in its study was carried out at that laboratory.

Measurements—tail end.

From bend of uterus to termination	.15 mm.
Tail width at bend of uterus	.14 mm.
Tail end of vitellaria from end	.12 mm.
Tail end from beginning of ovary	1.35 mm.
Width of ovary near tail end	.02 mm.
Width of vitellaria opposite tail end of ovary	.02 mm.

Head end.

Tip of head end from bend of uterus	.13 mm.
Width of worm across bend of uterus	.12 mm.
End of vitellaria at bend of uterus from tip end	.15 mm.
Vas deferens seen alongside vitellaria through much of its course, width of one column of uterus near anterior end, wide part	.02 mm.
Width of adult worm	.60 mm.
Length, probably 5 or 6 inches	
Egg	.005 mm.

Atalostrophion promicrops nov. sp.

On March 31, 1914, portions of a worm of the same genus as *A. sardæ* were found on the gills of a large Jew fish, (*Promicrops guttatus*) at the New York Aquarium. It was only discovered after the body had been disposed of, so that unfortunately the branchial cavity was not examined as it should have been, for no doubt more worms would have been found under the mucous membrane there.

One portion of worm found was about two inches long by .60 mm. wide. It was more muscular than that found in *Sarda sarda* and not so thin and delicate. It contained the same organ; the uterus, however, only showed a single tube filled with eggs. This, too, is a new species and will be fully described when further material is secured. I would in the meantime propose the name *Atalostrophion promicrops.*

The eggs are about of the same size and color as in *A. sardae* and the body, although thicker, is about .60 mm. in width.

Since the present paper has been in press, I have had occasion to examine a number of fish fresh from the sea, among

them two snappers, (*Priacanthus cruentatus*) whose death was certainly caused by *Diplectana*. The gills were infested by them, literally in thousands; in such numbers as I had never seen before. I have thought the fact of such importance that it should be recorded in connection with the above paper.

G. A. Mac.

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THE ASSIMILATION OF CHOLESTEROL AND ITS ESTERS.

By J. HOWARD MUELLER.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

(Received for publication, June 9, 1915.)

Although it is now a generally accepted fact that the cholesterol of the body has its origin in the food, and not, as has been held by some investigators, in an actual synthesis within the body, there is yet no very definite information as to the mode of absorption, or the form in which it is assimilated. It was with a view to clearing up these two points that the work represented in this paper was undertaken. The presence in the body of the free cholesterol, an alcohol, together with its esters of the higher fatty acids, oleic, palmitic, and stearic, makes it desirable to get a better understanding of the relations of these two forms in order to approach more nearly a solution of the problem of their physiology.

That cholesterol, when fed, is actually absorbed, and that the absorption is followed by a rise in blood cholesterol, has long been established. Jankau[1] found that when fed to rabbits and dogs, only a part could be recovered from the feces, although he was unable to recognize an increase in the blood. This was presumably due to the very imperfect methods of analysis available at that time. Pribram[2] demonstrated an increase in blood cholesterol after feeding, and believed that the increase was in part at least due to free cholesterol, since the serum from such animals showed an increased inhibitory power toward the hemolytic action of saponin, the free cholesterol only being active as an inhibiting agent. Kusumoto[3] also found that only a part of the cholesterol of the diet could be recovered from the feces. Gardner and coworkers[4] have shown definitely that feed-

[1] Jankau, L., *Arch. f. exper. Path. u. Pharmakol.*, 1892, xxix, 237.

[2] Pribram, H., *Biochem. Ztschr.*, 1906, i, 413.

[3] Kusumoto, C., *ibid.*, 1908, xiv, 411.

[4] Dorée, C., and Gardner, J. A., *Proc. Roy. Soc., Series B*, 1908, lxxx, 212; 1909, lxxxi, 109. Fraser, M. T., and Gardner, J. A., *ibid.*, 1909, lxxxi, 230; 1910, lxxxii, 559. Gardner, J. A., and Lander, P. E., *Biochem. Jour.*, 1913, vii, 576; *Proc. Roy. Soc., Series B*, 1913–14, lxxxvii, 229.

ing cholesterol, either free or as esters, results in an increase in the blood content both in the free and combined forms. Their work has been done on dogs, cats, rabbits, and chicks, using in part at least, the digitonin method of Windaus[5] for analysis, and there seems to be no reason to question their findings or conclusions.

Since most fatty material which is absorbed goes into the body through the lymphatics by way of the thoracic duct, it seemed probable that cholesterol would also be absorbed in this way because of its solubility in such material, and insolubility in aqueous media. Consequently a series of experiments was made on dogs for the purpose of determining whether or not a marked increase of cholesterol could be obtained in the chyle following a meal rich in cholesterol.

A thoracic duct fistula was produced in each case by ligating the external jugular vein at a point 2 or 3 cm. distal to the entrance of the duct, the innominate vein proximal to the duct, the subclavian vein, and several other small veins which enter the segment thus cut off. The jugular vein was then cut near the distal ligature, and its proximal end sewed into the corner of the incision. Chyle could be collected from a fistula so produced, by means of a cannula, or by allowing it to drop directly into a test-tube when the dog is standing up. Unfortunately the chyle coagulates rather readily and could not be made to flow for more than about twenty-four hours. Consequently it was usually practicable to make but a single experiment, or at most two, on each dog. I wish here to express my thanks to Prof. W. G. MacCallum for his kindness in doing these operations.

The plan followed was to allow the dog to recover from the etherization in the course of three or four hours, then to give the test meal, allowing the dog to eat voluntarily when possible, and at other times feeding first 10 to 15 grams of cooked lean meat in order to stimulate the digestive organs to normal activity, and then giving the desired material by stomach tube. A 10 cc. sample of chyle was taken either before or at the time of feeding, and afterwards at varying intervals, and a comparison of the cholesterol content of the specimens made by means of a modification of the Windaus method. The details of this method I

[5] Windaus, A., *Ztschr. f. physiol. Chem.*, 1910, lxv, 110.

have given in another place.[6] The ether extract of the chyle was obtained by mixing the sample with anhydrous sodium sulphate, allowing to dry, and grinding fine in a mortar, then extracting thoroughly with ether in a continuous extraction apparatus. A description of the experiments follows.

Experiment I.—Immediately after the completion of the fistula a sample of chyle was collected. Four and one-half hours later the dog was fed the yolks of four eggs mixed with a small amount of raw meat (20 to 30 gm.). This meal, although not analyzed, contained probably about 1 gm. of cholesterol, of which 90 per cent or more was free.[7] The material was eaten voluntarily by the dog. Table I shows the details of the experiment and analysis. The last column, headed "per cent," gives here, and in the succeeding tables, the per cent of the total cholesterol which is in the uncombined form.

TABLE I.

Dog 1.

Fistula Completed at 1 p.m.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
p.m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
1.00	13.4	0.0049	0.036	0.0088	0.066	35.29
*5.30	15.2	0.0060	0.039	0.0100	0.066	37.62
7.30	9.6	0.0059	0.062	0.0115	0.119	33.91
8.30	10.0	0.0088	0.088	0.0207	0.207	29.83
9.30	9.8	0.0111	0.113	0.0270	0.276	28.32

* Time of feeding.

Experiment II.—This experiment was the second carried out on Dog 2, the first being reported as Experiment IV. The test meal consisted of 30 gm. of lean meat and three raw eggs, with which was thoroughly mixed 0.5 cc. of oleic acid. A small aliquot of this was analyzed, and the whole found to contain 0.88 gm. of cholesterol, of which 88.15 per cent was free. Owing to the fact that the digitonin method gives low values for free cholesterol in the presence of much oily material, the amount of free cholesterol was probably something over 90 per cent of the total cholesterol. The dog ate the food voluntarily. The results follow.

[6] Mueller, J. H., *Jour. Biol. Chem.*, 1915, xxi, 23.

[7] Mueller, *loc. cit.*

TABLE II.

Dog 2.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
a. m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
7.30	10.5	0.0043	0.041	0.0097	0.092	30.83
*8.30						
11.30	9.3	0.0058	0.062	0.0164	0.177	23.94
p. m.						
12.30	10.4	0.0067	0.065	0.0162	0.155	29.54
1.30	11.2	0.0062	0.055	0.0159	0.139	28.35

* Time of feeding.

Experiment III.—In order to see whether pure free cholesterol dissolved in some neutral solvent would also be assimilated in the same way, this dog was fed first a very small piece of lean meat, which he ate, and was then given by stomach tube 0.75 gm. of cholesterol (Merck) dissolved in about 10 cc. of cottonseed oil and emulsified in 250 cc. of water by means of white of egg. No trouble was experienced, either in this or in the following experiments where a similar procedure was followed, in the dog's vomiting the liquid so administered.

TABLE III.

Dog 3.

Fistula Completed at 11.30 a.m.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
p. m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
*3.15	11.4	0.0049	0.043	0.0092	0.081	34.67
5.15	10.7	0.0076	0.071	0.0164	0.153	31.70
7.15	11.0	0.0083	0.076	0.0193	0.175	30.28
11.15	10.8	0.0145	0.134	0.0415	0.385	25.82

* Time of feeding.

Experiment IV.—In order to investigate the absorption of cholesterol esters it was necessary to resort entirely to the use of synthetic compounds, fed by the method used successfully in the case of free cholesterol in Experiment III. The esters used, the palmitate and oleate, were prepared

according to the method recommended by Hürthle,[8] by heating cholesterol with five times its weight of the acid to be used to about 200° for two hours, and purifying the esters so produced by alcohol, in which they are very difficultly soluble. In this experiment the dog was given first 17 gm. of lean meat, which had been found to contain about 0.015 gm. of cholesterol, mostly free, and then by stomach tube 1.5 gm. of cholesterol palmitate, dissolved in cottonseed oil, and emulsified in water with egg white. The cottonseed oil was found on analysis to contain in 1 cc., 0.0010 gm. of material precipitated by digitonin before saponification, and 0.0016 gm. after saponification of the filtrate from this. This substance is probably the so called phytosterol, which evidently exists as an ester, as well as free, although I am not aware that this point has been brought out before. It is closely related chemically to cholesterol, and is converted into it when fed to animals.[9] The results follow.

TABLE IV.

Dog 2.

Fistula Completed at 11.30 a.m.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
a. m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
11.30	7.8	0.0013	0.016	0.0029	0.037	30.19
p. m.						
*3.40						
5.30	9.8	0.0025	0.025	0.0039	0.040	38.46
6.40	9.9	0.0026	0.026	0.0038	0.038	40.62
7.40	9.9	0.0027	0.027	Lost		

* Time of feeding.

Experiment V.—The chyle obtained in this experiment was very bloody a small vein having evidently been left opening into the jugular. This accounts for the relatively high proportion of free cholesterol found, the cholesterol of dog blood being in the neighborhood of 80 per cent uncombined. The dog was given the same test meal as the preceding one; *viz.*, 1.5 gm. of cholesterol palmitate dissolved in cottonseed oil and emulsified in water.

[8] Hürthle, K., *Ztschr. f. physiol. Chem.*, 1895–96, xxi, 331.

[9] Fraser and Gardner, *Proc. Roy. Soc., Series B*, 1909, lxxxi, 230.

TABLE V.

Dog 4.

Fistula Completed at 11.15 a.m.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
p. m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
4.30	10.1	0.0082	0.081	0.0036	0.035	69.83
*5.15						
7.15	10.0	0.0116	0.116	0.0048	0.048	70.73
9.15	11.7	0.0129	0.110	0.0067	0.057	65.87

* Time of feeding.

Experiment VI.—Cholesterol oleate was substituted in this experiment for the palmitate, the same amount being used.

TABLE VI.

Dog 5.

Fistula Completed at 1.30 p.m.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
p. m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
*4.30	10.7	0.0026	0.024	0.0045	0.042	36.36
6.45	9.8	0.0093	0.095	0.0133	0.136	41.13
8.45	9.7	0.0065	0.067	0.0100	0.103	39.41
a. m.						
12.30	10.0	0.0067	0.067	0.0106	0.106	38.73

* Time of feeding.

Experiment VII.—Chyle was still running from the fistula used in the preceding experiment the next morning, and another experiment was undertaken on the same dog, using this time 1.5 gm. of cholesterol palmitate. Unfortunately only one sample of chyle could be obtained after feeding, the fistula becoming permanently thrombosed.

TABLE VII.

Dog 5.

Chyle.		Free cholesterol.		Cholesterol esters.		Per cent.
Time collected.	Amount.	Amount.		Amount.		
a.m.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	
9.00	9.9	0.0032	0.033	0.0042	0.043	43.42
*9.30						
p.m.						
12.30	9.4	0.0073	0.078	0.0106	0.113	40.84

* Time of feeding.

The figures quoted leave little doubt but that cholesterol is absorbed in the chyle together with other lipoidal material. It is of course not certain that no cholesterol is taken up by the blood stream directly. Unfortunately no determinations were made on the blood itself during the course of these experiments. It might be argued that an increase in the cholesterol of the lymph would not necessarily indicate an absorption through the lacteals, but that if the absorption were primarily by way of the blood stream, an increase would naturally follow in the lymph of the thoracic duct, since the general lymph has its origin ultimately in the blood plasma. Such a supposition, however, is not borne out by the figures, the increase in some, at least, of the experiments being too great to be accounted for on such a basis. The dogs used were all fair sized; one (No. 2), which was afterward bled to death for a different purpose, yielded about a liter of blood. Of this, perhaps 750 cc. were composed of plasma. In order to account for the increase which was shown in Experiment II on this dog, over 0.7 gram of cholesterol would have been required for this amount of plasma alone, without taking into consideration the blood remaining in the dog, the lymph and intercellular fluid, or the tissues themselves, which have been shown to share in the increase.[10] Since by actual analysis, less than 0.9 gram of cholesterol was fed in this experiment, and since it has been repeatedly shown that there is not a complete absorp-

[10] Gardner and Lander, *Biochem. Jour.*, 1913, vii, 576.

tion of cholesterol, much that is fed appearing in the feces either as such or as coprosterol, its reduction product, it is obvious that the increase shown by the chyle could not be explained on such a basis. In other experiments the rate of increase would have required a still larger quantity of cholesterol.

It is evident, further, from the figures, that the intestine itself is a most important site for the conversion of free cholesterol into its esters or *vice versa*. This is probably the first direct evidence of this process by any particular tissue, although it has, of course, been known that such changes take place in the organism. In those experiments in which a meal was given containing almost entirely free cholesterol, the increase is evident, not only in the free cholesterol of the chyle, but also, and to about twice the amount, in the combined cholesterol. On the other hand, when cholesterol almost entirely in the combined form is fed, there is likewise an increase in both fractions, about one-third being attributable to free cholesterol. Evidently during the absorption, the trend of the process is to preserve the ratio between free and esterified cholesterol which is normal to the chyle, and either an esterification or a hydrolysis may take place, according to the character of the material supplied. It is odd that in all the experiments in which a preponderance of free cholesterol was fed, there appears to have been a slight decrease in the relative amount of free cholesterol in the total, while in two of the experiments in which the esters were fed (IV and VI), the reverse is true. The significance of this fact, if any, is not apparent.

In Experiment II it was attempted to show that the formation of esters from the free cholesterol might be due to the liberation, during the digestion, of free fatty acids. By adding oleic acid to the food it was thought that a considerably greater increase in esters might be shown than in Experiment I, the two being in other respects similar. The results did not in any way bear out the hypothesis. It is possible, of course, that the amount of oleic acid used (0.5 cc.) was too little to give a decided effect.

In Experiment III, the finding of such a large amount of cholesterol in the last sample of the chyle eight hours after feeding, which contained also considerably more oily ether extract than the preceding ones, may be explained in part by slow absorption owing to the weak condition of the dog, but probably

more by the blocking of the chyle in the intestinal lymphatics, following the temporary closing of its exit. The chyle never ran continuously, but coagulated in the orifice of the duct soon after each sample was taken. As stated above, it was always possible to start the flow again without any difficulty by inserting a cannula until about twenty-four hours after the operation, when permanent clotting usually took place.

The experiments on cholesterol palmitate were not, on the whole, as satisfactory as those on free cholesterol and cholesterol oleate, in all of which very marked absorption was indicated. Experiment IV is almost entirely negative, even the apparent increase in free cholesterol in the second sample being probably due to a false value for the first determination, since figures of this kind were not obtained in any other case. The extremely small size of the first sample renders this likely. It may be noted, however, in this connection that this is the only ambiguous figure in the whole series, the work taken altogether indicating that the modification of the digitonin method of analysis used here is remarkably accurate, even for much smaller quantities of material than has been generally believed.

Experiment V, in spite of the large amount of blood present, shows a distinct increase in cholesterol content, while the increase shown in Experiment VII is equal to that obtained in the feeding with free cholesterol.

CONCLUSIONS.

1. Cholesterol is readily absorbed from the intestinal tract through the chyle and may be recognized in the contents of the thoracic duct.

2. When cholesterol is fed entirely in the free condition, a portion is esterified during the absorption and appears so in the chyle, and *vice versa*. Hence either in the intestinal contents or in the wall of the intestine, processes of esterification or of hydrolysis may take place which result in the absorption of the cholesterol in approximately the same proportion of free to ester found normally in the chyle.

3. This ratio seems not to be affected by the presence of small quantities of fatty acids in the food.

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EOSINOPHILIC INFILTRATION OF THE PANCREAS

HENRY A. RILEY, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, New York City)

The case which is the subject of this report possesses interest in that as far as can be ascertained, it is the only case on record which presents an eosinophilia limited to the pancreas alone and

existing in that organ without discoverable cause and independent of a generalized eosinophilia.

Before describing the histological characteristics of the organ involved, it may be well to consider briefly the cause of generalized and local eosinophilia. The main authority consulted is the exhaustive study on general and localized eosinophilia by E. Schwartz in the *Ergebnisse der allgemeine Pathologie*, 1914, Vol. 17, Part I.

General Eosinophilia: Drawing his facts from many sources, Schwartz places the normal limits of the eosinophilic cells in the circulating blood between 2 per cent. and 4 per cent. in adults, 2 per cent. and 5 per cent. in children. Race, sex, age, temperature of the environment, pregnancy and parturition seem to bear no direct influence upon the eosinophile cells. The condition of nourishment appears to exert some influence, starvation reducing the number of cells, which, however, rapidly returns to normal upon the restitution of sufficient nourishment.

Splenectomy or ligation of the splenic vessels is followed by an immediate decrease in the number of cells, then a rise to a supernormal figure and then a slow drop to a subnormal figure which is then maintained.

The effects of many and varied toxic agents have been studied. Those which produce an increase are antimony, CO, CO_2 and pilocarpine. Hæmotoxic substances seem to exert no constant effect upon the number of eosinophiles. Anaphylactic shock is regularly followed by a marked and protracted eosinophilia. This phenomenon is considered to be the explanation of the eosinophilia observed in asthma, hay-fever, many of the dermatoses and certain obscure intestinal phenomena which have been held by many to be due to localized anaphylactic conditions.

Disturbances of the glands of internal secretion do not seem to exert any constant effect upon the eosinophiles except in the thyroid; hypothyroidism being fairly constantly accompanied by an increase, hyperthyroidism by a decrease in the number of eosinophiles. Certain nervous diseases are accompanied by an eosinophilia, among which may be named epilepsy, especially in periods immediately following active seizures, dementia præcox, paranoia, and many psychoses of various origins.

The conditions which produce the most striking examples of eosinophilia are those associated with parasites. The site of the infection does not seem to influence the blood picture, for similar extreme examples are found in all of the groups of parasites whether they exist in the intestinal canal, the parenchymatous organs, the urinary system, the muscles, or the blood. They differ markedly in their eosinophilic properties, however, some of the parasites being unable to stir up any response, while others produce such a profound effect that the eosinophilic cell may occupy an almost exclusive position in the composition of the white elements of the blood. In general, higher values are observed in children than in adults. Those parasites which are

FIG. 1. Low power picture showing islands of large size and eosinophilic infiltration of the surrounding tissues.

capable of passing into encysted form gradually show a diminishing influence on the eosinophiles. If, however, the contents of the cyst gain access to the blood stream either by rupture, or by aspiration and injection, an immediate eosinophilic response results. Of the intestinal parasites, the *Ankylostoma duodenale* stands at the head in its ability to produce an eosinophilic response, values as high as 80 per cent. having been recorded. When it is considered that at the same time there is a marked rise in the total number of leucocytes, it can readily be seen that enormous numbers of new cells have been added to the circulating blood. Malaria in its chronic forms has been known to produce 20 per cent. of eosinophiles.

Certain of the infectious diseases present instances of moderate increases in the number of eosinophiles,—rheumatic fever, scarlet fever, vaccinia, the post-critical period in pneumonia when the eosinophiles which have heretofore been completely absent from the blood reappear. In gonorrhea, especially with the incidence of complications, a moderate increase in the number of eosinophiles has been observed, while extreme values of 25 per cent. have been recorded. Amœbic dysentery has also shown high figures, 47 per cent. having been reported in one case.

In tuberculosis we find the eosinophiles playing a rôle of some prognostic importance for they disappear from the blood in cases of advancing involvement by the tubercle bacillus and reappear only when the reparative processes of the body have succeeded in outstripping the ravages of the disease.

The list of dermatoses with which eosinophilia has been associated is a long and formidable one. It is an almost constant accompaniment of urticaria, eczema, prurigo, iodide and mercury erythemata, and all vesicular and bullous diseases. Psoriasis shows an eosinophilia only inconstantly, but it is invariably present in the dermatitis exfoliativa which often follows psoriasis.

A general eosinophilia is also seen accompanying many of the granulomata and neoplasms. Prominent among these is Hodgkin's disease where figures up to 28 per cent. may be encountered. Curiously enough, higher values may be met with after *x*-ray treatment, percentages up to 68 per cent. having been reported. General sarcomatosis and carcinosis may produce a generalized eosinophilic response even to 45 per cent. in cases of the latter. Lymphosarcoma may also produce a general blood-reaction, but to a less degree.

Local Eosinophilia: The eosinophiles are found widely scattered throughout the body under physiological conditions, both in the fluids of the body and also in the tissues. In the hæmolymph glands they are more numerous than in the ordinary lymph glands. They are said to be absent from the mucous membrane of the œsophagus and stomach but are present in considerable numbers in that of the intestine. A diet mainly of meat or one containing many irritating components is said to increase the number of the eosinophiles in the mucosa. During the digestive periods the number in the spleen increases, while in fasting periods only a few can be found. When present they are found only in the pulp. There are no recorded investigations as to their presence in the salivary glands or the pancreas.

They exist in only moderate numbers in the walls of the respiratory

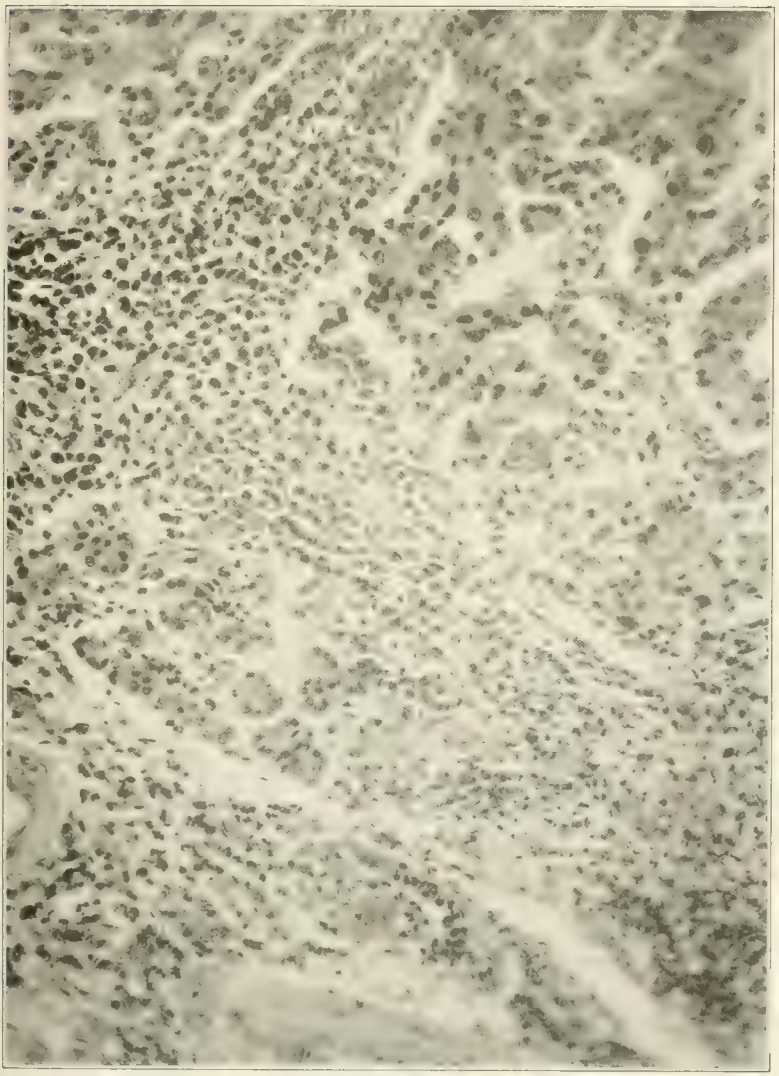

FIG. 2. High power of same showing disordered structure of the islands and the infiltration of the surrounding tissues by eosinophiles.

tract, but are said to present accumulations about the mucous glands of the bronchi.

They may be found in varying numbers in the thymus increasing up to the seventh month and then receding.

In lesions of the gastro-intestinal tract they may be materially increased in number, being found in catarrhal gastritis, atrophic gastritis and especially in tissues showing neoplastic changes. They are supposed to bear a direct relation to the increased mucous secretion of the disordered mucosa rather than to the processes of disintegration. In some forms of appendiceal inflammation they are quite numerous. These accumulations in the mucosa of the intestinal tract are attributed to local immunity reactions comparable to the generalized eosinophilia associated with anaphylactic phenomena.

They are present in the myocardium of cases dying during the course of diphtheria. They are not found in cases proving fatal prior to the seventh day of the disease. Attempts to explain them on the basis of anaphylaxis resulting from the injection of antidiphtheric sera have not been successful, for they have been found as frequently in those who have been and those who have not been treated with serum. They are not present in increasing numbers in comparison with the number of serum injections, and they bear no relationship to the clinical severity of the case. They are found closely gathered about the smaller blood vessels but not in the region of blood extravasations nor near the foci of degeneration.

They are inconstantly present in the pus arising from non-specific inflammations, but in the pus from gonorrheal processes in any site they are abundantly present, increasing in direct relation to the duration of the process until they may make up almost the entire bulk of the suppurative material. In the nasal and bronchial secretions from asthma and hay-fever they are very numerous and may in these instances be largely mononuclear in character. In asthma it has been noted that large numbers of the leucocytes found in the urine may be eosinophiles. The fæces in cases of intestinal helminthiasis may contain large numbers of eosinophilic leucocytes. Pleural exudates often show increasing numbers of eosinophiles as the inflammatory process recedes.

The parasitic lesions of the skin show a local eosinophilia, the cells being arranged either in groups or scattered diffusely through the tissues. Often they are gathered about the smaller blood vessels. In cases of infection with *Trichinella* the eosinophiles are not strictly limited to the immediate vicinity of the parasite, but they may be found throughout the interstitial inflammatory tissue. In lymph node involvement by *Filaria* the eosinophiles may be found in large numbers occupying both the lymph sinuses and the lymph cords. The lymphatics may also be found to contain great numbers of eosinophiles. *Balantidium coli* is also capable of producing a marked eosinophilia, the infected areas of the colon containing many of these cells.

The dermatoses in a similar manner show a local eosinophilia, the tissues surrounding and underlying the lesions often being tightly packed with eosinophiles. The skin diseases capable of producing a local eosinophilia are identical with those which are responsible for the similar generalized blood reaction.

The local eosinophilia about neoplasms is often of considerable importance. This question presents many obscure and puzzling features for no one constant factor can be found exercising any marked influence upon this specialized tissue reaction. One form of tumor may in one site be accompanied by a marked eosinophilia only to find a similar reaction completely lacking in a similar tumor in another region, and one form of tumor in a given region may supply many eosinophiles, whereas a different tumor in the same tissue will show none. In general, however, it may be stated that those tissues which are exposed to the greatest irritation are those in which the eosinophiles are the most numerous.

The carcinomata are fairly regularly accompanied by a moderate eosinophilic infiltration, the uterus being the tissue which is capable of furnishing the greatest numbers of these cells. They are also present to a lesser degree in sarcomata of all types. They are usually infrequent in the hypernephromata. Benign growths may also show an eosinophilia, but less regularly and to a slighter degree, than the malignant growths.

The lesions of periarteritis nodosa show regularly a diffuse admixture of eosinophilic cells.

CASE REPORT

The child whose pancreas is the subject of this paper was born on the 25th of March of this year at the Nursery and Child's Hospital. It was the last of fourteen pregnancies, one of which terminated in a still-birth; all the others however, including this one were normal in all respects. The child was living until labor began, but as it was a very large child, weighing over ten pounds and being a breech presentation with a long difficult labor, extraction was protracted and the child was born dead. There were no external abnormalities, no stigmata of syphilis, and all the organs were found in an approximately normal condition. The pancreas was grossly quite normal. The cause of death was ascertained to be a fracture between the third and fourth cervical vertebrae, consequent upon forcible extraction in the breech position. No suspicion of anything pathological was aroused in the routine microscopical examination except for a slight degree of degeneration in the parenchymal cells of the liver, kidneys and adrenals, until the pancreas was reached, where a most unusual and interesting condition was found. This change affected all the constituents of the pancreas, the islands of Langerhans, the interstitial tissue and last and least the acinar tissue. The same condition existed in all parts of the organ examined.

The islands were enormously increased in size and almost visible to the naked eye. They were much distorted and their usual orderly internal structure was much obscured. They were made up chiefly of two types of cells: (1) A cell which comprised the main bulk of the island and was large with a granular deep staining cytoplasm and an ill-defined cell outline. The nuclei differed much in size, some were very large indeed and presented a clear protoplasm, a definite chromatin network, and in some cases a distinct nucleolus. The cytoplasm of the cells in many areas resembled a syncytium. These cells suggested in many of their properties neoplastic cells. (2) The second type of cell was much less numerous and was arranged principally about the periphery of the islands. It had a small darkly staining nucleus, more or less oval in outline, in which no chromatin network could be made out. Its cell outline was somewhat more definite than that of the preceding cell, and its cytoplasm took a pinker stain. Occasional eosinophiles could be seen in among the cells of the island. In the spaces which surrounded the capillary loops could be seen numbers of beautifully granulated eosinophiles, which were in greater part multilobular, although mononuclear cells were present in fair numbers. In the connective tissue of the capillary loops the eosinophiles seemed to be surrounded by delicate connective tissue strands. The connective tissue of the pancreas presented the most extraordinary eosinophilic infiltration, the entire tissue having been simply converted into eosinophilic tissue, each cell, in many instances, surrounded by delicate connective tissue fibrils. Between the eosinophile cells could be seen occasional connective tissue cells and only very infrequently a polynuclear leucocyte. The tissue was so densely infiltrated with eosinophiles that it was difficult to demonstrate any particular condensation about the blood vessels. The capillaries were engorged and the lining endothelium seemed enlarged and swollen, but only occasional eosinophiles could be found within the capillaries. There was a large amount of free eosin-staining granular material present in the section. Scattered here and there singly and in small clumps between the cells could be seen long needle-like crystals pointed at each end and somewhat thicker at the

middle, which took a pink stain, and seemed to be typical Charcot-Leyden crystals. The acinar tissue was much reduced in amount and the acini were in large part isolated from one another by a uniform increase in the amount of inter-acinar connective tissue which appeared oedematous and was loaded with eosinophiles. The majority of the acinar cells appeared quite normal but occasional vacuolated cells and indeed entire degenerating acini could be found. The eosinophile cells did not invade the acini.

Attempts to clear up the etiology of this condition have been without success. Tissue stained by the Levaditi method and examined for spirochaetae has not disclosed any of these organisms. Efforts to find bacteria in preparations stained by the Gram method and with Giemsa's stain have also been unsuccessful.

The question of the origin of the eosinophiles, whether by emigration from the blood vessels or by local production will be left open.

It is equally impossible to give any definite opinion as to the underlying cause for this peculiar picture, but it would seem that it was associated with the islands of Langerhans. The appearance of the cells of the islands indicated that some unusual process was in course there and it is conceivable that some abnormality in their metabolism may be responsible for the eosinophilic infiltration.

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AN EXPERIMENTAL STUDY OF BLOOD GLYCOLYSIS. THE EFFECTS OF THYROID AND ADRENAL EXTRACTS AND PHLORHIZIN ON GLYCOLYSIS IN VITRO.

BY GEORGE M. MACKENZIE, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

(Received for publication, June 17, 1915.)

Efforts to localize and measure glycolysis have been very unsatisfactory, especially since the theory of Cohnheim[1] concerning glucose destruction by the combined action of extracts of muscle and pancreas has been shattered by Levene and Meyer.[2] Levene and Meyer showed that the sugar was not really oxidized in such mixtures, but converted into other forms which fail to reduce copper. Hydrolysis of the resulting fluid restores the glucose with its original power of reduction. Nevertheless, Levene and Meyer admitted that glycolysis could be brought about by leucocytes, and many others have shown that if blood be incubated for several hours the amount of glucose remaining is distinctly less than in the freshly shed blood. This oxidation of glucose in the blood is probably, however, of very slight physiological importance.

It is known that glycosuria appears after the injection of certain materials into the body, and it was thought desirable to determine, if it could be demonstrated by using the ordinary blood glycolysis as a test, whether these substances have any effect upon the action of a sugar-splitting enzyme. It was realized that other explanations of their action are generally accepted and that their application to a somewhat artificial process of glycolysis outside of the body might be criticized. Nevertheless, the experiments were made to learn whether, in any degree, they could act to inhibit a glycolytic process.

[1] Cohnheim, O., *Ztschr. f. physiol. Chem.*, 1904, xlii, 401; 1906, xlvii, 253.
[2] Levene, P. A., and Meyer, G. M., *Jour. Biol. Chem.*, 1911, ix, 97.

EXPERIMENTAL.

Study of Blood Glycolysis. Effects of Thyroid and Adrenal Extracts and Phlorhizin on Glycolysis in Vitro.

Method.

Dogs were used in all the experiments. The animals were etherized and either a carotid or femoral artery exposed. A cannula, with rubber tube attached, was inserted in the artery and the blood drawn directly into a sterile bottle containing glass beads. The blood was defibrinated and 20 cc. portions pipetted off and placed in sterile 150 cc. Erlenmeyer flasks which were plugged with sterile cotton. The amount of reducing substance in the blood drawn was determined immediately; in most of the experiments duplicate portions were used. Table III shows the difference found in the duplicate portions of the same blood. The other portions, after the substances being studied for their effect on glycolysis had been added, were placed in the incubator at 38° C. for varying periods of time and then the reducing substance determined. For comparison of the rate of glycolysis an untreated portion of the same blood was, in each case, incubated along with the treated portions.

In all the determinations the protein was precipitated with colloidal ferric hydroxide and sodium sulphate[3] and the reducing substance found by the method of Bertrand.[4]

Inasmuch as only comparative values were sought rather than an accurate determination of the normal blood sugar level of the dog, no effort was made to avoid the effects of excitement, struggling, and ether. The figures, therefore, are somewhat higher than those of Shaffer,[5] Embden, Lüthje, and Liefmann,[6] who eliminated these factors as completely as possible. Shaffer's results varied between 0.02 and 0.065 per cent, Embden, Lüthje, and Liefmann's between 0.057 and 0.088 per cent. Macleod[7] gives the figures of several observers which vary between 0.086 and 0.207 per cent. His own vary between 0.079 and 0.205 per cent.

[3] Rona, P., and Michaelis, L., *Biochem. Ztschr.*, 1908, vii, 329. Macleod, J. J. R., Diabetes, London, 1913, 28.

[4] Bertrand, G., *Bull. de la Soc. chim. de Paris*, 1906, xxxv, 1285.

[5] Shaffer, P. A., *Jour. Biol. Chem.*, 1914, xix, 297.

[6] Embden, G., Lüthje, H., and Liefmann, E., *Beitr. z. chem. Phys. u. Path.*, 1907, x, 265.

[7] Macleod, *loc. cit.; Jour. Biol. Chem.*, 1913, xv, 497.

In all the experiments sterile conditions were maintained as far as possible. Cultures were taken from the incubated blood in the first few experiments. A few of these were contaminated but most of them were sterile. To determine the effect of contamination upon the rate of disappearance of reducing substance, duplicate portions of the same blood were incubated for the same period of time, one under aseptic conditions, the other without sterile glassware and without any effort to avoid contamination. The results are shown in Table I. The loss of reducing substance is tabulated here, and in other tables that follow, as percentile glycolysis, which expresses the ratio of the amount of sugar lost during incubation to the amount originally present; *i.e.*, $\frac{\text{sugar lost per 100 cc.}}{\text{sugar originally present per 100 cc.}} =$ percentile glycolysis.

TABLE I.

Comparative Values for Blood Glycolysis under Sterile and Non-Sterile Conditions.

Experiment.	Glucose before incubation.	Condition of blood during incubation.	Duration of incubation.	Glucose after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
I	0.130	Sterile	3	0.073	43.8
		Non-sterile	3	0.076	41.5
II	0.130	Sterile	3	0.075	42.3
		Non-sterile	3	0.073	43.8
III	0.100	Sterile	3	0.074	26.0
		Non-sterile	3	0.075	25.0

From this it appears that the difference in the amount of glycolysis taking place in sterile and contaminated blood during a period of three hours' incubation is within the limits of experimental error, and, therefore, that failure to maintain sterile conditions probably has little or no effect on the amount of glycolysis occurring within a period of three hours.

In order to determine how much variation there might be in the rate of glycolysis in several portions of the same blood taken at the same time and kept under the same conditions, three 20 cc. portions of the same blood were incubated in separate flasks and the percentage of sugar then determined. The results are shown in Table II.

TABLE II.

Variations in the Amount of Glycolysis in Several Portions of the Same Blood.

Experiment.	Glucose before incubation.	Portions.	Duration of incubation.	Glucose after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
I	0.100	*A*	3	0.069	31.0
		B	3	0.074	26.0
		C	3	0.073	27.0
II	0.130	*A*	3	0.070	46.1
		B	3	0.073	43.8
		C	3	0.075	42.3

This indicates that different portions of the same blood under identical conditions show very slight variation in the rate of glycolysis, and this factor, therefore, does not explain irregularities in the results after adrenalin, thyroid extract, and phlorhizin have been added to the blood.

Table III is given to show the amount of experimental error by the methods used. The greatest difference found was about 3 per cent.

TABLE III.

Percentage of Blood Sugar in Two Portions of the Same Blood.

	Portions.	1	2	3	4	5	6	7	8
Percentage of blood sugar	*A*	0.110	0.099	0.174	0.176	0.100	0.175	0.131	0.133
	B	0.112	0.096	0.172	0.175	0.101	0.173	0.129	0.135

Effect of Fresh Thyroid Extract upon Blood Glycolysis.

The results of experimental work upon the relation of the thyroid secretion to carbohydrate metabolism are somewhat conflicting. Macleod[8] after reviewing the work that has been done concludes that the thyroid glands, in so far as carbohydrate metabolism is concerned, have no direct influence whatever. The results which some observers[9] have obtained, tending to show that the thyroid furnishes a substance which lowers the assimilation limit for dextrose, he ascribes to parathyroid effect.

Clinical observations on the blood sugar, glycosuria, and dextrose assimilation limit in cases of hyperthyroidism and hypothyroidism

[8] Macleod, Diabetes, London, 1913, 104.

[9] Eppinger, H., Falta, W., and Rudinger, C., *Wien. klin. Wchnschr.*, 1908, xxi, 241; *Ztschr. f. klin. Med.*, 1908, lxvi, 1; 1909, lxvii, 380.

seem to indicate, however, that the gland does have some effect upon carbohydrate metabolism. When the thyroid is supplying the blood with abnormally large quantities of its secretion, the blood sugar rises, glycosuria often appears, and the dextrose assimilation limit is lowered. The reverse is the case in myxedema. If the thyroid secretion could be shown to contain an antiferment it might act simply by preventing the sugar-splitting ferments of the blood and tissues from oxidizing the carbohydrate. This would result in hyperglycemia, glycosuria, and lowered tolerance for glucose. The following four experiments were done to determine whether such an antiferment could be extracted from the gland.

Thyroid extract was prepared by extirpating the entire gland of a dog, and grinding it up in a sterile mortar with sterile normal salt solution or sterile Ringer solution, care being taken to exclude the parathyroids in so far as possible. The amount of extract used, whether 5 or 10 cc., represented one entire gland. The extract was added to the blood taken from another animal and the mixture incubated. Controls with the amounts of Ringer solution or saline used in the extract were incubated at the same time. For comparison one portion of untreated blood was incubated for the same period. The results are shown in Table IV.

TABLE IV.

Experiment.	Glucose before incubation.	Mixtures incubated.	Time of incubation.	Glucose after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
IX	0.098	Blood 20 cc. + 10 cc. fresh thyroid extract	3	0.060	39.7
		" " " " " Ringer solution	3	0.054	44.8
		" " " untreated	3	0.080	18.4
X	0.175	" " " + 5 cc. fresh thyroid extract	3½	0.096	42.8
		" " " " " Ringer solution	3½	0.106	38.7
		" " " untreated	3½	0.110	37.1
XI	0.175	" " " + 5 cc. fresh thyroid extract	2½	0.132	24.6
		" " " " " Ringer solution	2½	0.138	21.1
		" " " untreated	2½	0.145	17.1
XII	0.152	" " " — 5 cc. fresh thyroid extract	3	0.094	38.1
		" " " " " " 0.9 per cent sodium chloride	3	0.088	42.1
		Blood 20 cc. untreated	3	0.103	32.2

From these results one cannot conclude that the thyroid secretion contains any substance which materially affects the rate of glycolysis *in vitro*. In each case there is apparently some acceleration of

glycolysis both in the flask containing the extract and in the flask containing the Ringer or physiological salt solution. In Experiment IX the acceleration is well marked, in the others, slight. Possibly this is due simply to the dilution, because in the experiment in which the glycolysis was most marked the dilution was greatest. The experiments do not, of course, settle the question of whether or not the thyroid gland furnishes an antiferment which inhibits glycolysis; it is possible that the gland contains such a substance but that it is not extracted by the methods used. To the extent, however, that the conditions of glycolysis *in vitro* are similar to glycolysis in circulating blood, they furnish an argument against the hypothesis that the thyroid gland affects carbohydrate metabolism by inhibiting ferment action.

Effect of Adrenalin on Blood Glycolysis.

Blum,[10] fourteen years ago, showed that subcutaneous or intravenous administration of adrenal extract causes glycosuria. Since then this work has been abundantly confirmed, and it has been shown that adrenalin regularly causes a hyperglycemia. Experiments of Pollak[11] showed that adrenalin produces glycosuria even after the resection of the splanchnic nerve, while the work of Claude Bernard[12] and Eckhard demonstrated that, after such resection, puncture of the floor of the fourth ventricle is not followed by glycosuria. The adrenalin is, therefore, thought to act by stimulating the peripheral sympathetic nerve endings in the liver. Moreover, it causes the hyperglycemia even in animals whose livers have been rendered glycogen-free by starvation. This is thought to be due to the production of sugar from protein. Despite these explanations of the action of adrenalin in the production of hyperglycemia and glycosuria, it seemed to be worth while to determine whether or not the adrenal secretions contain a substance acting as an antiferment. With this possibility in mind the following experiments were done.

[10] Blum, F., *Deutsch. Arch. f. klin. Med.*, 1901, lxxi, 146.

[11] Pollak, L., *Arch. f. exper. Path. u. Pharmakol.*, 1909, lxi, 149.

[12] Bernard, C., Leçons sur la physiologie et la pathologie du système nerveux, Paris, 1858.

Parke, Davis and Co. adrenalin was used. Quantities varying from 1 to 5 cc. were added to 20 cc. portions of blood and the mixtures incubated for varying periods. The sugar content of the blood was determined immediately after drawing the blood, and in the incubated portions immediately after the expiration of the period of incubation. A portion of untreated blood was also incubated for comparison. The results are shown in Table V.

TABLE V.

Experiment No.	Adrenalin 1:1,000.	Glucose before incubation.	Duration of incubation at 38° C.	Glucose after incubation.	Percentile glycolysis.	Glucose in untreated blood after incubation.	Percentile glycolysis in untreated blood.
	cc.	*per cent*	*hrs.*	*per cent*		*per cent*	
IX	5	0.098	3	0.039	60.2	0.080	18.3
X	5	0.173	$3\frac{1}{3}$	0.102	41.0	0.119	31.1
XI	5	0.175	$2\frac{1}{2}$	0.150	14.2	0.145	17.1
XIII	5	0.152	3	0.064	57.8	0.103	32.2
XIV	1	0.100	$2\frac{1}{2}$	0.049	51.0	0.037	63.0
XIV	2	0.100	$2\frac{1}{2}$	0.043	57.0	0.037	63.0
XIV	3	0.100	$2\frac{1}{2}$	0.044	56.0	0.037	63.0
XV	2	0.174	5	0.124	28.8	0.120	31.0

In Experiments IX and XIII the blood with added adrenalin showed a definite acceleration of glycolysis as compared with the untreated blood. In Experiment IX about three times as much reducing substance disappeared from the adrenalin blood as from the untreated blood, in Experiment XIII a little less than twice as much. In the other experiments the difference in the percentile glycolysis between the treated and the untreated blood was so slight as to be almost within the limits of experimental error.

The results are so irregular that one is not justified in concluding that adrenalin contains any substance which either accelerates or inhibits blood glycolysis. The explanation of the apparent acceleration in two experiments is not clear.

Effect of Phlorhizin on Blood Glycolysis.

It is believed that the glycosuria after administration of phlorhizin[13] results from the power of phlorhizin to render the kidney more permeable to sugar in the blood, thus lowering the threshold for sugar excretion. There is usually a hypoglycemia. Beyond the fact that phlorhizin renders the kidney more permeable to

[13] For a full discussion and complete bibliography of phlorhizin, see Lusk, G., *Ergebn. d. Physiol.*, 1912, xii, 315.

glucose, little is known of its action. The possibility of its acting as an antiferment suggested itself.

Phlorhizin in varying amounts was dissolved in sodium bicarbonate solution, warm Ringer solution, and blood serum, and then added to blood which was incubated for varying periods. Dry phlorhizin also was added to blood in one experiment. The amount of reducing substance lost during the incubation of these mixtures was compared with the loss of reducing substance from an untreated portion of the same blood taken at the same time and incubated for the same period. The results are shown in Table VI.

TABLE VI.

Experiment No.	Phlorhizin.	Solvent.	Glucose before incubation.	Duration of incubation at 38° C.	Glucose after incubation.	Percentile glycolysis.	Glucose in untreated blood after incubation.	†Percentile glycolysis in untreated blood.
	mg.		*per cent*	*hrs.*	*per cent*		*per cent*	
III	20	1 cc. 2 per cent sodium bicarbonate	0.102	2	0.077	24.5	0.083	18.6
IV	100	5 cc. 2 per cent sodium bicarbonate	0.152	3	0.103	32.2	0.93	38.8
V	100	5 cc. 2 per cent sodium bicarbonate	0.151	3	0.107	29.1	0.102	32.4
VIII	100	5 cc. 2 per cent sodium bicarbonate	0.144	2½	0.095	34.2	0.126	12.5
IX	50	5 cc. Ringer solution	0.098	3	0.162	39.6	0.80	18.3
X	50	5 " " "	0.174	2⅓	0.132	24.7	0.119	31.2
XIII*	60	10 cc. serum	0.152	3	0.185	26.5	0.103	32.2
XV	100	Dry	0.174	5	0.142	18.4	0.120	31.0
XV	50	5 cc. Ringer solution	0.174	5	0.109	37.0	0.120	31.0

* In this experiment deduction was made for the amount of sugar contained in the 10 cc. of serum used to dissolve the phlorhizin. The phlorhizin was partly in solution and partly in suspension.

In six of the nine experiments the difference in the percentile glycolysis between the phlorhizin and the untreated blood was not more than 7 per cent. This difference is too slight to justify an inference that the phlorhizin affected the rate of glycolysis. In Experiment VIII there was apparently an acceleration of the glycolytic process; in Experiment XV apparently a retardation; in Experiment IX instead of a loss during the period of incubation there was an increase of 39 per cent in the amount of reducing substance. I cannot give any satisfactory explanation for this. From these results one cannot conclude that phlorhizin has any effect upon the rate of glycolysis.

CONCLUSIONS.

1. Blood glycolysis *in vitro* during a period of three hours' incubation proceeds at practically the same rate under sterile conditions and when no effort is made to prevent contamination.

2. Fresh thyroid extract, adrenalin, and phlorhizin do not contain any substances which have a constant effect upon the rate of blood glycolysis outside of the body. No evidence of the presence of an antiferment was found.

It is a great pleasure to express my indebtedness to Professor W. G. MacCallum, under whose direction this work was done.

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ON LIGATION OF SPLENIC VESSELS AS A SUBSTITUTE FOR SPLENECTOMY IN BLOOD DISEASES*

ALSO A CONTRIBUTION TO THE PATHOLOGY OF THE INFARCTS OF THE SPLEEN

BY ABRAHAM TROELL, M.D.
DOCENT OF SURGERY, STOCKHOLM, SWEDEN

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York)

THE removal of the spleen—an operation which is said to have been already performed during the Middle Ages (Carstens[9]) and which nowadays without question plays the most important rôle in splenic surgery—has in the last few years been gaining a great deal as far as its indications are concerned. For, according to the experience of unquestioned authorities, it has given favorable results in certain blood diseases, particularly morbus Banti and pernicious anæmia. Banti himself as well as others[a] gives reports of cases of the former disease which, operated on in an early stage, have completely recovered and have been doing quite well as long as four years after the splenectomy. As to the pernicious anæmia certainly a perfect health has not yet been established in any case in regard either to the general condition or to the blood picture of the patient, but an improvement has been noted, in some instances so direct and striking that it could hardly be due merely to one of the ordinary fluctuations in the run of this disease.[a] Furthermore it has been shown, both by experiments and through clinical experience, that, from a physiological point of view, the spleen is not an absolute necessity for the organism. As far back as 1841 Bardeleben[10] found this to be true in animals, and numerous observations made upon patients who have undergone splenectomy have shown them to be in good health many years after, Adelmann's[18] case, for instance, 23 years. Thus there is no doubt that the operation as a matter of principle is justifiable.

* These investigations were carried out in the laboratory of Dr. W. G. MacCallum (Professor of Pathology, College of Physicians and Surgeons, Columbia University, New York) during the winter semester 1914–1915, and I am very much indebted to him, not only because he has granted me a place in his laboratory, but also for the great interest he has always shown in my work during this time and through which he in every way made it possible for me to undertake it and bring it to an end.

[a] Moynihan and Upcott, Michelsson, K. Ziegler, Poppert, Dahl, Stapelmohr, Roblee, Moffitt, Rystedt, etc.

However, it involves a considerable immediate risk. Every one who has had to operate upon individuals greatly debilitated by blood diseases, as well as he who on account of such a condition has refrained from operating, is aware of this. Splenectomy is particularly dangerous where adhesions from perisplenitis increase the difficulty in removing the organ (if not making it altogether impossible) and increase the chances for fatal bleeding.[b] In the 33 cases of splenectomy for pernicious anæmia that Moffitt has recently collected and published 8 fatalities had happened in direct connections with the operation. In Banti's disease the operative mortality is estimated at 50 per cent. (K. Ziegler). Indeed, the real mortality for all operations performed in these diseases is not small.

With this fact in view the thought has occurred to me that it might be possible to get similar results or at least nearly similar results for these patients through a simpler and less dangerous operation than splenectomy. I mean by *ligation of a larger or smaller number of splenic vessels*. Since in morbus Banti and pernicious anæmia a complete elimination of the function of the spleen has a favorable effect upon the organism, then a partial lessening of its function ought to affect the metabolism beneficially too. For a long time, and for good reasons, we have accepted an analogous way of thinking in regard to another ductless gland, viz., the thyroid, in its relation to Graves's disease. Such a clinician as Halsted, whose experience and critical way of thinking are well known, speaks in favor of this operation. And on the whole the clinical experience has proven this unmistakable, even though there has been a theoretical doubt as to the effectiveness of the operation because of the great number of anastomosing vessels, giving a very rich vascular distribution to the gland.[c]

I have searched through the literature for notes on the ligation of splenic vessels for the purpose mentioned above. The idea of this operation seems in fact so natural, that its earlier origin in the minds of others is not at all surprising. On the contrary, it is strange that it has not as yet led to the thorough investigation of its presumptive value.

In the chapter on "Surgery of the Spleen" in the large surgical text-book of Keen, Moynihan and Upcott make the following statement concerning partial ligation of the hilum of the spleen: "It has been shown experimentally that reduction of the blood-supply by ligation of some of the arterial branches entering the spleen results in atrophy of the organ, and as long as veins are left intact necrosis will not occur." However, I have not been able to get any further

[b] *Cfr.* Fischer, Roblee and others.

[c] *Vid.* for instance, Landström: Morbus Basedowii, Nord. med. arkiv., i, 1907.

information as to this investigation as no one of the papers referred to by them contains the original statement and they do not give reference to authority. Most of the other accounts in the literature concern the ligation of all splenic vessels. In order to find a method of operation in traumatic splenic bleedings—ruptures, gunshot wounds, etc.—Girgolaw and Sheldon ligated the whole hilum of the spleen in dogs, a method first advanced by Jonnesco.[58] Both undertook the operation on three dogs, all of which died from intoxication by the decomposition-products of the spleen. To prevent this, Sheldon has followed Pirone's[58] suggestion and sewed up the large omentum all around the spleen, thus hoping to get a new blood-supply to the organ Nevertheless, the animals died after 12 to 48 hours, and at the autopsy the spleen was found necrotic throughout. The statements of Long and Lanz are based upon clinical experience, each one reporting a case of ligation of the splenic artery in "fixed floating spleen." In such a disease, not menacing the life of the patient, Lanz advises "in complicated cases," instead of splenectomy, that ligation of the splenic artery be performed as it is less dangerous but equally effective. In his case, he claims to have found clinically, half a year after the operation, a "complete disappearance" of the spleen (?). He ends his paper as follows: "If, in a case of pernicious anæmia, the chances should be in one way or another unsafe as to splenectomy, then—according to my experience—the ligation of the splenic artery might be recommended." Finally, Foà gives a quite opposite suggestion. Starting from the fact that he has seen experimental tuberculosis in the liver of guinea-pigs cured after tying the splenic vein, which organ at the same time atrophied, he thinks it might be well to ligate the splenic vein in those cases of Banti's disease in which the spleen is not removable.[d]

The suggestion of ligating a part of the splenic vessels in order to produce a reduction in the functions of the organ leads at first to the following questions:

How will the organ be affected anatomically? and what will be the result on the function of the spleen?

I have tried to get information on these subjects by experiments on animals. Having ligated one or more vessels in the splenic hilum, I have kept the animals alive a longer or shorter period, after which, through another laparotomy, I have controlled the anatomical effect. I have given particular attention to the presence or absence of infarcts in the spleen and to signs of post-operative infection. As far as the physiological side of the subject is concerned, I intended from the beginning to get light on it by counting the blood-corpuscles before the operation as well as a couple of weeks afterwards, both in the afferent and efferent splenic vessels and in a vein in some other part of the body

[d] According to Michelsson, ligation of the splenic vessels in fixed malaria-spleen has been suggested by Clement-Lucas and carried out by Meierowitsch (and Wyman?). Michelsson—who does not give the exact reference for any of these authors—does not think this operation to be preferable to splenectomy in malarial spleen.

(the ear). The difference between the number of blood-cells in the vein and in the artery of the spleen ought, if the spleen plays any rôle in regulating the quantity of blood-cells in the general circulation, to differ in these two instances, and from that difference I expected to be able to judge whether the function of the spleen has been retarded or not. Yet I may at once admit that my chances in this respect were not very great as to a positive result, since the investigations so far made on the functions of the spleen have led to contradictory conclusions. There has been attributed to the spleen, among other things, a rôle of formation of blood-cells as well as one of destruction of them. Clinicians to-day generally hold that the spleen, as far as the blood is concerned, has to destroy old, useless erythrocytes and to regulate the throwing into the circulation of new elements from the bone-marrow and other blood-forming organs.[e] There are, however, even recent investigations (Morris) which are in favor of the theory that the spleen in adults, too, is a blood-forming organ.

There is one more detail in the arrangement of my investigations to be mentioned. For, in case of success in my attempt to produce what I in the first place expected, an infarct, I had to take into consideration a fairly important point. Against the use of such an operation on human beings the objection could be made that the production of a large necrotic piece of tissue in the abdominal cavity might mean a great risk of secondary infection. Realizing this, I infected three of the animals with ligated splenic vessels through the intravenous injection of staphylococci or coli bacilli one to one and a half weeks after the primary operation.

On account of space I do not give here a detailed report of my experiments (these will soon appear in a paper in the Swedish journal, *Svenska läkaresällskapets handlinger*). However, I wish to make a few technical remarks. Before the operation I made a blood test from the ear vein of the animal and counted the number of blood-cells. All the operations were performed under ether (between 10 A.M. and 2 P.M., the animals were fed at 5 P.M.; thus a possible leucocytosis of digestion should have played about as little rôle in one case as in another). The spleen was exposed by an incision parallel to and just below the costal margin. Through a slight pulling on the stomach wall the spleen was brought outside the abdominal wound and then protected with warm saline cloths. As much care as possible was taken to avoid direct handling and squeezing of the spleen as well

[e] See Johnston, Pearce (and collaborators), Thévenot, Hedenius, Moffitt, Dahl, A. Meyer (thorough paper on the relation of the spleen to the metabolism), King, Roblee and others.

as pulling and traction upon its vessels; this in order to exclude error in the blood-counts. After having located the topography of the vessels, I first punctured a vein and then an artery with a fine suture needle. From each vessel I took blood in a pipette for white and red blood-cells and mixed as usual with Hayem's solution and $^1/_3$ per cent. acetic acid. At my first operations I also made smears for differential counts of the white blood-cells (Giemsa stain). Finally, the number of vessels I wanted to ligate were tied with a linen thread, the spleen was replaced in the abdomen, the wound closed and the animal left to recover. All counts of the blood-cells were made by myself, using a Thoma-Zeiss apparatus; 100 squares for the red cells; the whole chamber for the white being counted.

The operations were so far successful that peritonitis never occurred. Healing followed without any trouble, the animals recovered very soon after the operation and, with some few exceptions, seemed to do very well.

I performed in the beginning a series of operations on 12 animals. From 1 to 5 each of the arteries and veins in the splenic hilum were tied, always as many afferent vessels as efferent ones, and in that way, as a rule, at least one artery and one vein were left untouched; with the exception of one of the guinea-pigs where all of the vessels at the hilum of the spleen were tied.

The anatomical effect in the large animals (eight dogs and one cat) of this series was negative, so far as I could judge, in six instances but positive in three. Every one of the three guinea-pigs showed marked changes in their spleens.

In the six animals first mentioned I could not find any evident abnormality in the splenic tissue at the autopsies undertaken, 13 days to 2½ months after the operation. This was the fact even in those two cases (Nos. 2 and 6) which, having been quite well up to the time of death, died spontaneously three weeks after the operation. One dog (No. 8), however, showed, 10 minutes after the ligation, multiple infarction of the periphery of the spleen in an early stage (Fig. 5); one cat (No. 9) after five hours, showed a diffuse infarction of the organ; and another dog (No. 5) after 13 days, showed a small hemorrhagic infarct in the upper part of the spleen.

As to the guinea-pigs, the effect was more noticeable. The size of the spleen in each case was greatly reduced, in proportion to the number of vessels ligated. Thus the animal in which two arteries and two veins were left untouched at the operation, showed, 1½ months later, a spleen somewhat larger than that in which just one artery and one vein had been left; while in the guinea-pig with its whole hilum tied,

the spleen was smallest. Yet in these three animals there was a general reduction of the organ without any visible signs of infarction.

Thus the results of this series of operations in a way were not very encouraging in view of what was expected. Particularly striking was the fact that infarction in so many cases did not occur, though I sometimes ligated as many as 4 out of 5 large arteries and veins, and although I always found my ligatures all right at the autopsy. It is to be regretted that at these first operations I had not taken an exact measurement of the spleen and thus could not later on tell whether the size of the spleen in the larger animals had been reduced after the ligation of the vessels. In the guinea-pigs this was an easy matter, as the decrease was so marked.

Evidently, the cause of the non-infarction should be found in the anatomical arrangement of the vessels. And yet at first, I was unable to determine exactly how, due largely to the fact that the distinguishing of the different parts in the gastrosplenic ligament (which in dogs is very broad) had mostly become rather difficult because of adhesions and kinking after the ligation. The fact was made clear, however, through one of the animals last killed (Case 1). In this specimen it was easily seen that distal to the place of ligation (Fig. 1)—which is indicated on the photograph by a pin through a paper cross—there was a transverse, broad vascular anastomosis, establishing a communication between the periphery of the tied vessel and the non-ligated vessel nearest to it. (This was found in the artery as well as in the vein, though the figure shows only the vein which afterwards was filled with a 5 per cent. collargol solution in order to render it more visible; for the same purpose a small piece of paper was placed under it). The main reason for ligating the splenic vessels at a point situated so proximally in the hilum was a technical one. With the intention of doing a blood count from vein and artery, I wished as much as possible to avoid handling and squeezing the vessels which I was going to puncture and then ligate; otherwise a disturbance might easily have been brought about in the physiological differences which I figured to exist in the amount of blood-cells in the artery and the vein. Thus I chose proximal places because the technic became simpler in that way—yet the vascular lumina not even here being very great on the animals operated on.

When I started operating upon my first series of animals, I undertook it with the idea in mind which has been found in anatomical textbooks for a long time (*e.g.*, Henle) and according to which the arteries of the spleen do not communicate with each other, either before enter-

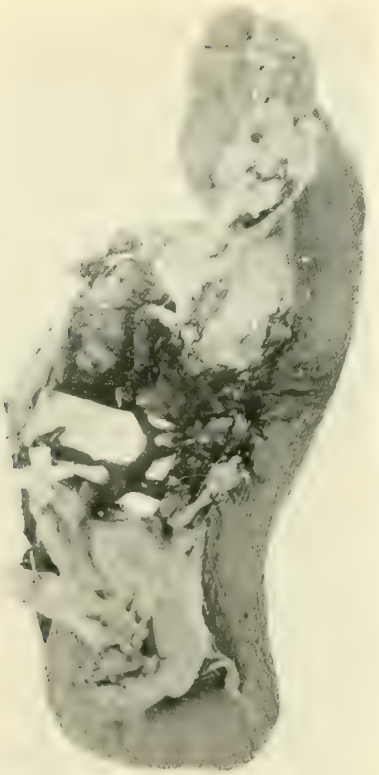

Fig. [illegible] Case I. Distal to the place of ligature, which is indicated by a pin through a paper ring. There is a transverse, broad vascular anastomosis, establishing a communication between the periphery of the tied vessel and the non-ligated vessel nearest to it.

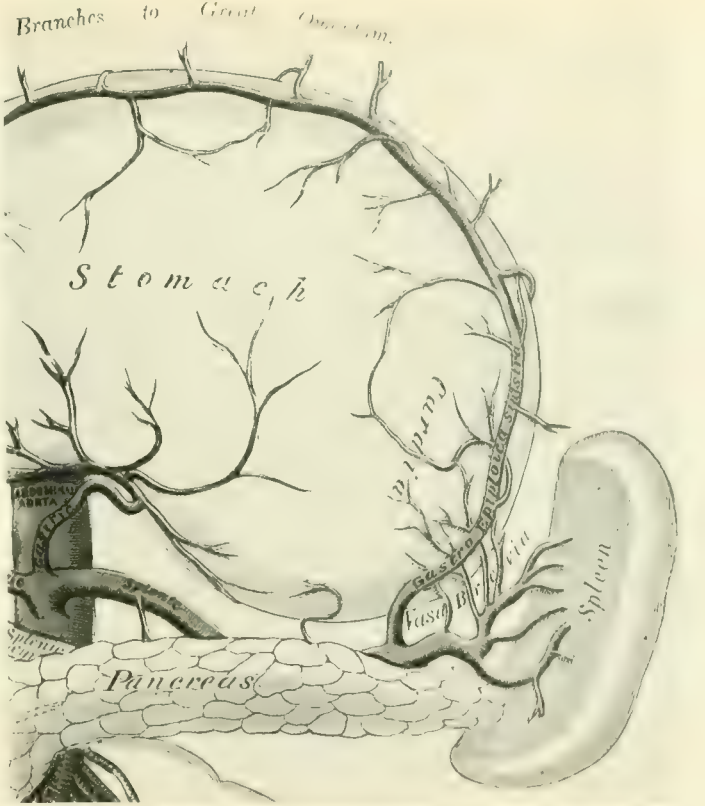

Fig. 2. Showing that the arteries of the spleen do not communicate with each other (from Gray's Anatomy).

Fig 3.

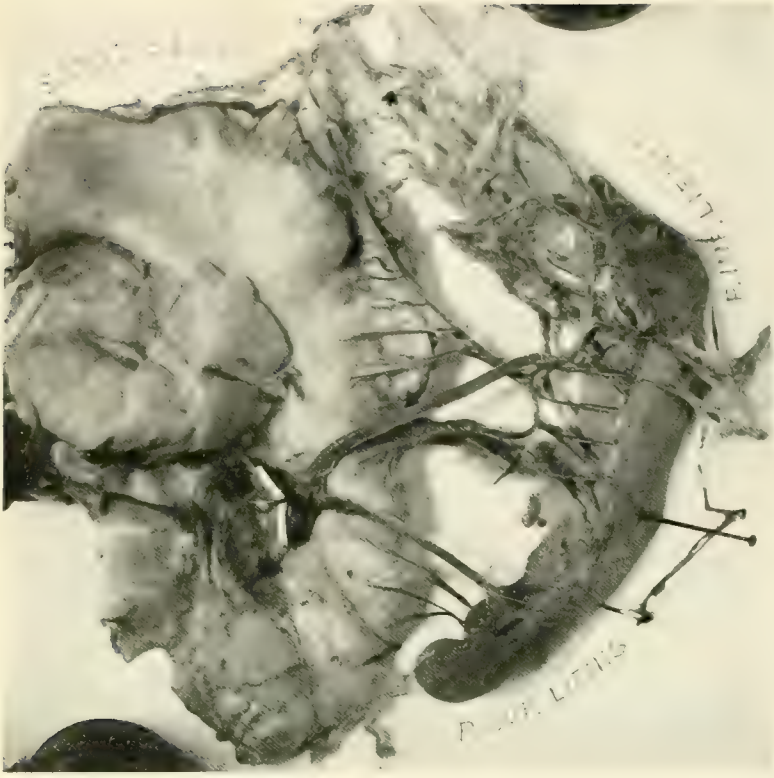

Fig 4.

Figs. 3 and 4.—From dog and man respectively. Showing the rich supply of transverse communication between the vessels of the spleen hilum; the anastomoses are located quite close to the concavity of the spleen.

FIG. 5. Case VIII. Ten minutes old, multiple hemorrhagic infarction, localized to the edges of the spleen.

FIG. 6. Case XXI. Three days old infarction of the lower and, to a less extent, upper poles of the spleen.

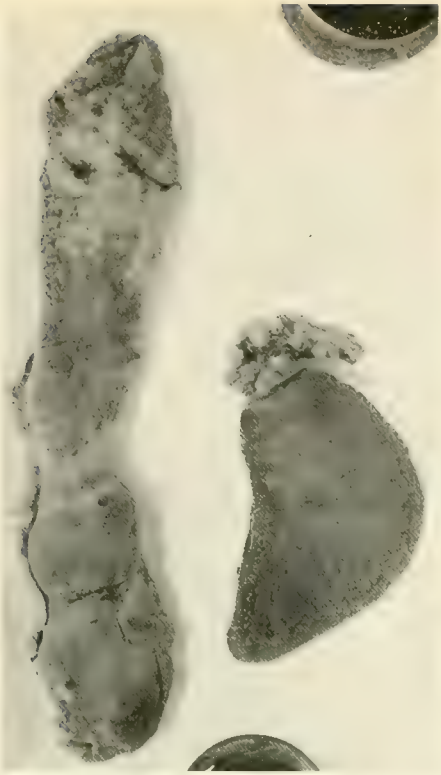

FIG. 7.—Case XX. Four and one-half weeks old infarcts, extended over the entire cross-section of the spleen. Case XVI. Spleen with its upper pole reduced to a pale shrunken infarct, 6 mm. long and 3 mm. deep; four and one-half weeks old.

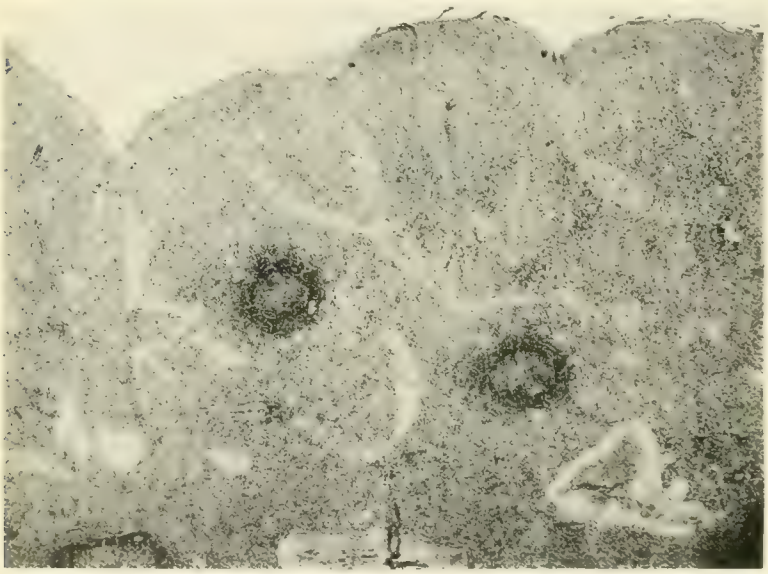

FIG. 8.—Case VIII. The microscopical appearance of a ten minutes old infarct; through an enormous over-supply of red blood-corpuscles the spleen cells are made more attenuated than usual, the malpighian bodies, as well as the trabeculæ, showing no visible changes.

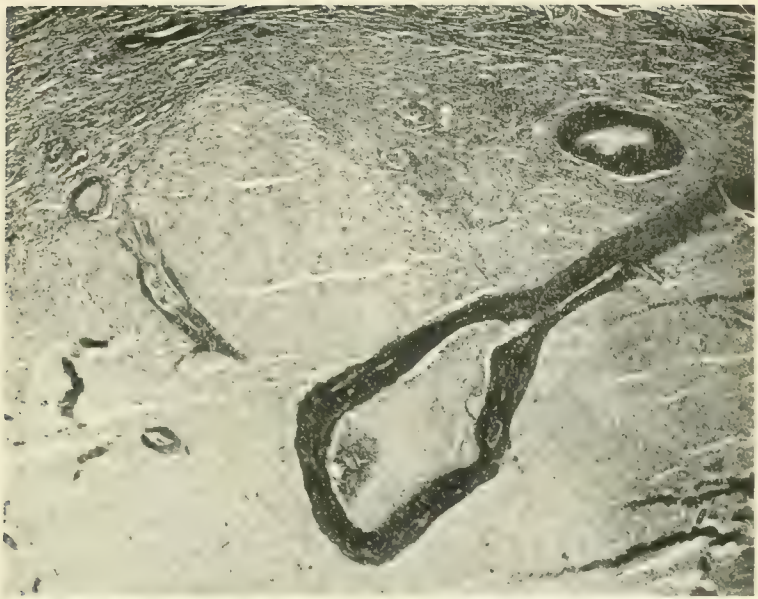

FIG. 9. Case XX. The histological picture of a four and one-half weeks old infarct, being in the state of organization; necrosis and, to a certain degree, reabsorption have begun. A part of the infarct remains in the shape of a central, hyaline eosinophilic mass, at the periphery of which there is a reaction zone rich in cells and pigment.

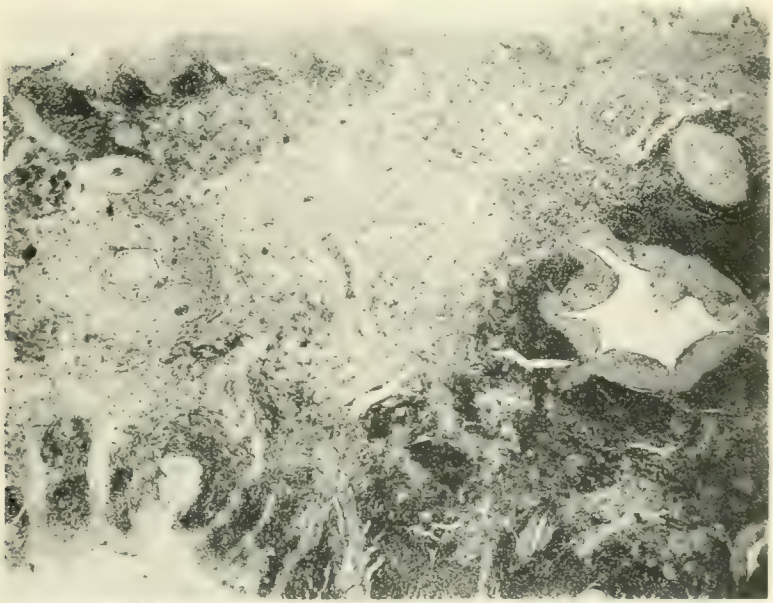

FIG. 10. Case XVIII. The histological appearance of a four and one-half weeks old organized infarct, consisting only of connective tissue rich in vessels, and to a great extent, sclerotic, containing pigment, intracellularly and extracellularly.

ing the organ or after (*cf.* Fig. 2, from Gray). I had no idea of the fact that anastomosing branches which exist between the splenic and the gastro-epiploic vessels could be so extensive as it afterwards appeared from the real effects of my ligations and as it was indicated from the findings in Case 1. Having stated this, however, I naturally felt very anxious to get reliable information on this point. Therefore I injected colored gelatine solution into the splenic arteries of fresh autopsy material, from dog as well as from man—stomach, spleen and pancreas being dissected out together.[1] There is no need of many words to emphasize how clearly the pictures (Figs. 3 and 4) bring out the rich supply of transverse communication between the vessels of the spleen hilum. The first photograph refers to the spleen of a normal dog, the second to that of a child one year old (the picture here seen was the same in principle in the other two human spleens which I examined for the same purpose). In the two cases shown in Figs. 3 and 4 the injection was performed at the point shown by the pin. After the injection, in order to show the details, all the veins as well as the peritoneal sheaths were dissected off. For the fixation 5 per cent. formalin was used. The specimens show, on the one hand, the presence not only of slight connections but also of very *wide and sturdy transverse communicating branches between the various vessels in the splenic hilum.* And, on the other hand, they present evidence that for the most part, *these transverse anastomoses are located quite close to the concavity of the spleen.* I have in many cases found them to be so distributed that one might reasonably speak of a marginal artery and vein, which is in communication with the spleen through short branches. In view of such a supply of vessels it is evident that, even without attaining an immediate and complete stoppage of the circulation, such as would be a necessary condition for infarction, it is quite possible to ligate several arterial branches, particularly by doing so at a sufficient distance from their entrance into the spleen.

By ascertaining the anatomical state of the vessels, a neglect of which explains the negative results of my first series of experiments, I found it very desirable to undertake a new series of ligations. In this series care should be taken, in order to cut off the blood supply to a certain part of the spleen, to place the ligature distal to the transverse branches extending between the individual arteries, or a supplementary ligation of the corresponding transverse anastomosing branches should be made. This second ligature should, therefore, be placed and tied in the direction

[1] For the spleen of the dog the gelatine was colored with Prussian blue, for that of the man with vermillion red.

of the ligamentum gastrosplenicum, at a right angle with the first ligature.

I carried out these ligations in a number of cases, a series of nine dogs. In some I ligated only arteries, in others also veins. In all—as well as in a guinea-pig—I made exact measurements of the spleen before the ligation, for the results previously obtained with the guinea-pigs operated on (Nos. 10–12) spoke in favor of the possibility that diminished blood supply and nutrition might bring about a general reduction of the organ without producing an infarct.[9] The ligatures I arranged so that I could not see any transverse branches lying distal to them or—and this latter was as a rule much more simply and quickly done—after ligation of, say, the lower half of the spleen hilum, I drew (Cases 20–22), with the aneurism needle, another ligature from the same point of departure, the centre of the hilum, straight out in the direction of and quite close to the concave surface of the spleen. The results of these operations were much nearer what I wanted than had been the case in the first series.

It may not be out of place here to touch upon the anatomical relations of the vessels *within* the spleen. *A priori* it would be quite conceivable that they might govern the manner in which the organ would react on shutting off a part of the circulation.

The finer structure of the splenic vascular system has for a long time been the subject of active study and discussion. The point in dispute, essentially, is the manner of the connection of the most diminutive arteries with the most diminutive veins: whether the thing takes place directly, by means of a closed system of tubes, as is usually the case elsewhere in the body, or whether it makes use of the intermediation of a system of lacunæ between the arteries and veins. The latter, if it exists, would in the minds of most investigators lack an at least uninterrupted wall, and consequently grant the blood the possibility of flowing out freely from the arterial capillaries into the splenic pulp before it accumulates in the smallest vein branches. Owing to the nature of the walls of the vessels, the blood would then be able to pick its way through the entire cellular structure of the spleen pulp in the same manner as, according to the picturesque description of Henle, a liquid would through a sand-hill. Very weighty reasons have been advanced in the last few years, in support of the view that the blood, on its way through the spleen, does not need to make use of any intermediary lacunæ, but continues proceeding from vessel to vessel, within vascular walls that form an uninterrupted connected whole, which nevertheless are so constructed that both red and white corpuscles are enabled to pass through them, by the process of diapedesis (Helly, Mollier, etc.; see also Weidenreich).

[9] The measurements were taken by placing a linen thread along the surface of the spleen, thus enabling me to obtain a precise value of the size of the entire organ in various directions.

The adherents of one view as well as those of the other are accustomed to base their opinions on now injection experiments, now a direct study of microscopical sections. But there are very slight prospects that either of these methods will ever show how an arterial capillary in the spleen ends, and how a venal capillary begins.

The method I have adopted to get at the answer to this problem is to *reverse the circulation* and then to judge from the microscopical sections whether the blood in the spleen circulates through an open or closed system. I proceeded, in the case of a dog, by applying Carrel's vessel clamps, and then severing one of the spleen's main arteries and one of its main veins. Then, by means of vessel suture, or with Crile's transfusion clamp, I united the proximal end of the artery with the distal end of the vein, and the proximal end of the vein with the distal end of the artery. After a period of 24 hours, at a relaparotomy, there was no noteworthy change in the macroscopical appearance of the spleen. Microscopically, on the other hand, it was quite clear that, in that portion of the organ which corresponded to the vessels affected, the tissue was remarkably full of red blood-cells. With the exception of the follicles (Malpighian bodies) which looked normal, the field was swarming with red blood-cells and very scarce of pulp tissue. Between the "operation area" of the spleen and its normal portion, there was a transition zone in which the frequency of blood-cells was not so pronounced. I shall not here enter into any further interpretation of these sections, as they cannot be considered as in any way representing a finished investigation. Yet it seems to me that they are fully compatible—and most compatible—with the assumption of a system of closed tubes in the spleen, in which the walls of the vessels in the finer branches on the venous side are more easily passed through than on the arterial side, so that, in the former part, under the influence of the more energetic blood current, it permits the passage of the blood-corpuscles to an unlimited number, spreads the Malpighian bodies to a greater distance from each other than obtains usually and fills the spleen pulp with red cells. The great frequency of blood-corpuscles even in the arteries belonging to the Malpighian bodies may be an indication of the presence of vessel walls all over, may be of very slight resistance. If the "reversed blood" from the vein branches reached very soon intermediary wall-less lacunæ, it could hardly have succeeded in getting so far back, *i.e.*, so far into the arterial side, that even the arteries of the follicles became full of red blood-corpuscles.

After this digression, I return to the cases operated on by me (Nos. 13–22), where the ligation of vessels gave a more positive result than I had previously obtained.

Post-operative peritoneal infection did not in this series occur, but two dogs (Nos. 14 and 21) died suddenly a short time after the operation, exactly as had been the case in the first series studied. The first dog (No. 14) presented a rather bad general condition before the operation. He died on the fifth day. No other organic change could be observed than the infarction in the spleen. The other dog who died (No. 21) was a rather large animal in fairly good general condition before the operation. He died three days after four-fifths of

his spleen hilum had been ligated, and on post-mortem examination showed no other abnormality than infarction of a large section of the spleen (Fig. 6). In these cases death may have been due to an intoxication produced by decomposition products of the spleen. Considering the cases together with the manner in which the guinea-pigs operated on reacted—including the two (Nos. 12 and 13) in whom the entire spleen had been disabled, none of them showed any effect on their general condition—we are obliged to note that various species of animals do not bear equally well such a trauma as is involved in the ligation of the vessels above indicated.

As for the changes produced in the spleen itself in the second series of operations, they consisted of an infarction of a greater or less portion of the organ (Cases 14–18, 20, 21), or in a simple reduction of the volume of the spleen without demonstrable infarcts (Cases 13, 19, 22). Most striking was the effect in Case 16: Before operation the length of the spleen was 13 cm., breadth 4 cm., circumference 6 cm.; 33 days after the upper half of the hilum had been ligated, the corresponding dimensions were, 7.4, 3.2, and 6.1 cm. respectively, and the upper pole of the spleen had been reduced to a pale, shrunken infarct, 6 mm. long and 3 mm. deep (Fig. 7).

From a pathological-anatomical view-point the infarcts are very interesting, which, including both series of operations, were produced in ten cases by ligation of a greater or less number of splenic vessels. They represent, taken together, a series of changes more or less pronounced, depending upon the length of time elapsing between the ligation and the animal's death. The age of the changes was from 10 minutes to 45 days.

Even gross-pathologically, it was easy to recognize those parts of the spleen which had been deprived of their normal blood circulations. They had the characteristic appearance of hemorrhagic infarcts: a wedge-shaped area on cross-section, with clear demarcations from the rest of the organ, with, after 10 minutes, a perceptible elevation and of bluish-black color (Fig. 5). Most often (Cases 8, 9, 14, 15, 17, 20, 21) they were multiple; in only two animals were they single (Cases 5 and 16). In five cases (8, 14, 15, 18, 21) there was noticeable a localization of the infarcts to the edges of the spleen (Fig. 5), or, chiefly to one pole or to both poles (Fig. 6). In four cases only one-half of the organ was affected, but in such a manner that the infarct (Cases 5 and 16, Fig. 7) or the infarcts (Cases 17 and 20, Fig. 7) extended over the entire cross-section, and did not limit themselves to the zones along the edges. The formation of infarcts in the centre

of the spleen with the periphery uninfluenced never came to my notice; in the two cases (Nos. 1 and 2) in which the spleen hilum was ligated in the centre, no effect at all was apparent.

It should, however, be noted that this negative result is stated for the condition several weeks after the operation; the effect may have had a different appearance earlier in the process. If a considerable part of the spleen's blood supply or discharge is suddenly cut off, the immediate effect will be a more or less pronounced (depending on the case) alteration in the corresponding tissue and its appearance. In Cases 14 and 21 (Fig. 6) there was recorded, even on the fifth (or fourth, respectively) day after the ligation of the vessels, a noticeable increase in the volume of the organ,[a] because of the increase in its blood content, congestion, infarction. A similar enlargement, not, however, one that was measured precisely, was observed in Case 9 five hours, and in Case 15 two hours after the operation. A few minutes after the ligation Case 9 showed multiple small protuberances on the convex surface, but five hours later, on the animal's death, the serous surface was entirely smooth and a general infarction of the organ had begun. Thus a certain adjustment of even rather extensive disturbances of the circulation can, it seems, in various ways be accomplished in due time. And it is not certain that if the animal in question had lived longer, a regular infarct formation would have developed in all the cases which showed infarcted portions at the post-mortem shortly after the vessel ligation. Case 22 gives further strength to this statement. Here, a few minutes after the ligation, there was observed a bluish-black color of the upper half of the spleen, which was in sharp contrast to the lower half of normal coloring. But when the animal was killed, four weeks after, no trace of an infarction could be found; there was merely a distinct reduction in the volume of the entire spleen.

As for the gross-pathological picture of the infarcts thus produced, it is noteworthy, finally, that an anæmic infarct could not be positively detected in any case. In, for instance, Case 18 (in which only the arteries were ligated) the macroscopic appearance of the infarction had a hemorrhagic color still a month later. From the microscopical picture it was not evident in a single case that an anæmic infarct had been produced. This, in view of what the experience shows as to human beings—but we most likely do not have many opportunities to see very fresh human infarcts—arouses our astonishment, though it agrees

[a] All these measurements were undertaken in exactly the same manner and under entirely uniform conditions, for which reason I regard it as quite proper to speak of the dimensions comparatively.

entirely with the earlier experimental results (Karsner and Austin, 1911, who produced infarction in the spleen and kidneys of dogs by introducing a number of plant seeds into the abdominal aorta). The growing pale of the infarct occurs sometimes earlier and sometimes later than the subsiding of its peripheral surface: in Case 17, ten days after the ligation, the infarcts were pale but not depressed; in Case 5, on the fourteenth day the color was hemorrhagic but the serous surface was retracted. Older infarcts had a depressed peritoneal surface (for example, Case 18).

Under the microscope it is easy to distinguish the effect of the circulatory disturbance in the spleen, both with regard to the blood accumulated and to the tissues of the spleen itself.

Already a short time (ten minutes) after stopping the circulation, there is a noticeable enormous oversupply of red blood-corpuscles (Case 8, Fig. 8). The spleen cells are much more attenuated than usual, particularly in a zone immediately under the organ's capsule of connective tissue. This hemorrhagic tissue is not so sharply contrasted with its surroundings as the macroscopically noticeable prominences on its serous surface might lead one to suppose, but merges very gradually into the tissue of normal blood content. Everywhere pigment is found very abundantly, both intra- and extra-cellularly, and often arranged in a thin wreath corresponding to the periphery of the smaller vessels (of the capillary vein?). The Malpighian bodies are in no place saturated with blood, nor in any way altered, nor are the trabeculæ.

After two hours, still more after five hours, it becomes emphatically evident that the frequency of blood-cells is much greater in the periphery than in the central portions (Cases 15 and 9). Certain spots do, however, present a thin zone of more normal spleen tissue, adjacent to the capsule. The outlines of the red blood-corpuscles begin to merge together and are difficult to distinguish. The follicles are no longer entirely free, but show, in various places near the periphery of the spleen, that the cells are pushed apart by red blood-corpuscles.

When the infarct is three days old, it has the appearance of an eosinophilic, structureless, almost hyaline mass, in which there are scattered a small number of well-stained cell nuclei, and also a number of poorly colored ones, as well as some fragments of nuclei (Case 21). The Malpighian bodies also show poorer coloring than usual, and the trabeculæ have in some spots been necrotized. This structureless area contrasts sharply with the adjacent spleen tissue, which is otherwise normal, but full of blood; the line of demarcation itself is made up of a thin—reactive—zone, rich in cells. All these alterations, however,

are not very pronounced at so early an epoch in the case of a small infarct. The contours of the blood-corpuscles are, to be sure, disappeared even in such cases, but the necrosis is not so far advanced. The blood pigment seems nicely placed in the protoplasm of the pulp cells. In Case 14, four days old infarction, also with minor infarcts, a considerable number of red blood-cells have retained their contours. Besides it is noticeable here that the lymphocytes, in this pathological process, are the cells that offer the most resistance to necrosis, and that a comparatively unaffected spleen tissue may continue to be present in a narrow zone close to the spleen's capsule.

In the next following stage, represented in my series of infarcts—the tenth day, Case 17—infarction has already passed beyond its climax. No grouping into follicles is any longer to be detected. Necrosis and karyorrhexis are as pronounced in the periphery as they are at the centre. Regressive processes appear within the infarct, and, at the same time, reactive processes from the adjacent parts are distinctly seen in the form of tissue, connected with the trabeculæ, and rich in cells and vessels. Areas are still found which by their eosinophilic nature indicate the presence, formerly, of red blood-corpuscles, but otherwise one has a predominant impression that most of the blood has been reabsorbed. In Case 5, a case with a very small infarct, four days later the destructive processes were decidedly less apparent than in the case described above.

The next stages I had occasion to study represented infarcts that were macroscopically shrunken and organized, 4½ weeks old. In two cases (Nos. 16 and 18, Fig. 10) I had the picture of connective tissue rich in vessels, and to a great extent sclerotic, and containing pigment within the cells as well as outside of them. The spleen's capsule was thickened and it did not run along in a straight line, but was wrinkled, apparently a consequence of the general reduction in the volume of the spleen brought about by the falling away of the specific spleen tissue. Very often, though, there was found, right under the capsule, a thin layer of common spleen tissue, rich in blood, before getting to the infarcts proper. In a third case of about same age (No. 20, Fig. 9), not all of the necrotic tissue had been reabsorbed, a rather considerable part of it remained in the shape of a central, hyaline, eosinophilic mass, at the periphery of which there began a reaction zone rich in cells and pigment.

It has already been remarked that the ligation of spleen vessels in two dogs operated on (Cases 19 and 22), as well as in all the guinea-pigs operated (Cases 10–13), did not produce any noticeable infarcts

after 1 to 1½ months, but rather a general reduction of the spleen's volume (to what extent such an effect was present in the dogs lacking infarct in the first series of operations cannot be determined, as I then did not make any precise measurements of the spleen). It has likewise been pointed out above that the anatomical reasons for this effect are to be found in the extensive anastomoses between the vessels in the hilum. Macroscopically, the diminution of the organ was not so great in the case of one (No. 19) of the dogs as in that of the other (No. 22), in which the length of the spleen had in four weeks been decreased by one-fourth of the original dimension. But for both of these cases the microscopic examination gave the impression of a diminished ratio of specific spleen tissue as opposed to connective tissue; necrosis could not be detected. In the guinea-pigs it was of interest (Cases 10 to 13) to note that a very considerable diminution of the organ had begun in spite of the fact that simultaneously the entire spleen had been enveloped in adherent omentum—showing that a subsidiary collateral circulation through the spleen capsule could not be developed and thus compensate the stoppage of the blood supply through the spleen hilum.

It would be no doubt valuable to conduct a long series of operations in which the ligatures should be intentionally so placed as to prevent the formation of infarcts, that is, without making the provisions recommended above, with regard to the transverse anastomosis between the vessels. It is not improbable that it might turn out that cutting off a considerable portion of the spleen's blood-vessels leads to a general, permanent reduction of the volume of the organ, without any infarct intervening. In that case this effect—so far as the conditions may be predicated of man—must seem more ideal than the direct production of infarcts, for the risk of secondary infection of the necrotizing infarcts here disappears. Yet there was not a trace to be found of any such infection in the spleen-operated animals whom I infected intravenously with pus bacteria (Cases 4, 5 and 17); this seems to indicate that spleen infarcts in dogs are not especially susceptible to infection. Besides it may be accepted for the present, that the rich supply of vessels in the individual cases, in the spleen, may make the projected anatomical reduction of the spleen tissue somewhat uncertain and difficult to previously calculate.

My earliest spleen operations I arranged so that I might if possible get some points on the influence of the stoppages on the functioning of the spleen. Various circumstances later prevented me from continuing in this intention. In the first place, I found contradictory results even in the very first counts of blood-corpuscles. Most of the animals did

give evidence of a larger supply of red blood-cells in the blood drawn directly from the spleen (vena lienalis), than in that found in the general circulation (ear vein) and in the vessels supplying the spleen (arteria lienalis) (Cases 1, 2, 4, 5 and 7). But in a few exceptional cases the opposite was true (Cases 3 and 6). The differences in the number of blood-corpuscles in vena and arteria lienalis were, furthermore, in some cases mighty small.[1] The prospects of obtaining any conclusions from a comparison between these first differences and those that I might have obtained by counting the blood-corpuscles a few months after the formation of infarcts, were consequently very slight. All the more so since, at this later epoch, the differences, depending on the extent to which the spleen had been infarcted, would have been less than the counts before the operations. To be sure it was also the negative result with regard to the infarct in the spleen, obtained from the animals first killed, that influenced me in giving up the blood-corpuscle counts entirely. It is yet not impossible that the accomplishment of a long series of operations and blood counts might give data of value, not only as to the formation of lymphocytes, but also on that of other blood-cells in the spleen. It would be important, in this connection, in view of the active anastomosis of the splenic vessels with the vasa gastro-epiploica, to make sure that such veins in the hilum be chosen as conduct, as far as possible, only blood from the spleen.

The task I had put before me in undertaking ligation of splenic vessels was to find out whether such an operation was an advisable substitute for splenectomy in certain blood diseases. The necessary conditions for its advisability would be, on the one hand, that a simple ligation should, in the case of the individuals concerned, bring about a rather considerable reduction of the spleen, anatomically and physiologically, and, correspondingly, an improvement in the patient's condition; on the other hand, that this should be obtainable at a slighter risk to the latter than is the case in splenectomy.

My investigations, ignoring for the present what they have taught me concerning the arrangement of the vessels in the spleen hilum and concerning the pathology of infarcts of the spleen, have shown that the cutting off of the spleen vessels in the case of the animals in question in a short time led to an anatomical reduction of the spleen specific

[1] Still more inconclusive were the differences with regard to the number of white blood-corpuscles. Their number alternated, for the nine animals first operated on, between 11,000 and 30,000. Differential counts gave a figure of at most 18 per cent. of mononuclears (about 300 to 450 leucocytes were counted in each case).

tissue. It is very likely that it also involves a reduction in the work of the organ. In other words, the effect of it may be qualitatively, but not quantitatively, equivalent to the effect of extirpating the spleen. As for the risk of a simple ligation of vessels, I have been able, in the animals I studied, to determine that the dangers of secondary infection from infarcts arising after ligation are at least not great, not even in the pyogenic condition.

In view of the high operative mortality of splenectomy, as well as of its impracticability in certain cases which have perisplenitis and adhesions, it may therefore be very reasonable to ask whether such stoppage should not be resorted to in pernicious anæmia and morbus Banti. The results I obtained concerning its effect are not applicable without further question to sick human patients. What its effect will be in such cases can only be determined by clinical experience. If favorable, it is possible that the advantages of this operation over splenectomy may not lie only in its applicability to inextirpable spleens. Its simpler technic—and presumably, therefore, its lower mortality in operation—might extend the operative indications in the case of the blood affections mentioned. For by having acquired an operative method that would grant temporary relief—and that is about all splenectomy has frequently done in these cases—we also have secured the prospect that a rather large number of patients suffering from chronic anæmias, whose complete cure has thus far been not attainable by our present therapeutic methods, may be successfully treated.

Note.—The substance of the above essay was communicated on February 19, 1915, to the regular weekly staff-meeting of the Department of Pathology of Columbia University; the number of the *Journal of American Medical Association* that appeared two weeks later, contained an article by Roblee on "Splenectomy in Primary Pernicious Anæmia" (March 9, 1915). Here it is stated that the ligation of the spleen vessels as a substitute for splenectomy has already been tried on six patients, but with a mortality of 66 per cent. However, the reference to authority is not given, and I have not been able to find in any of the authors cited (A. R. Steele, Harris, and Hertzog—except W. Mayo, on whose advice in the same periodical, in 1910, this mode of operation was tried) any original work such as was indicated.[1] I am therefore not acquainted with the details of these cases. It is surely not unimportant how great a portion of the spleen hilum was cut off, and less

[1] I have gone through the "Index Medicus" as well as the *Jour. of the Amer. Med. Ass.* from 1910 to 1914. Possibly Roblee's remark is based on some verbal statement of the operator in question.

so, whether not only the arteries, but also the corresponding veins were ligated. The difference in sensitiveness shown in my experiments on animals, as between guinea-pig and dog, may find a parallel in the similar relation between dog and man, in other words, man may be more vulnerable to such interferences than the dog, as the dog is more vulnerable than the guinea-pig. It should not be overlooked, that in both the animals in my second operation series (Cases 14 and 21) who died spontaneously, both the supplying and discharging vessels had been ligated. In the producing of an infarct it is of no advantage to ligate veins also, but for the removal of the decomposition products of the infarct, it is more desirable to afford as good an egress for the latter as possible, it means to ligate only a few of the spleen's arteries and none of its veins.

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AMYLOIDOSIS: (*a*) INCIDENCE. (*b*) REPORT OF CASE WITH EXTENSIVE AMYLOID-LIKE DEPOSITS IN ORGANS NOT SHOWING ANY OF THE CHARACTERISTIC REACTIONS

R. A. LAMBERT, M.D.

(*From the Pathological Laboratory of Montefiore Hospital and the Department of Pathology of the College of Physicians and Surgeons*)

(*a*) INCIDENCE

Among fifty-five autopsies performed at Montefiore Hospital during the past eight months nine cases of amyloidosis have been encountered, an incidence of 18 per cent. Chronic pulmonary tuberculosis was the cause of death in twenty-one cases, eight of which, or 38.1 per cent., showed a deposition of amyloid in various organs.

As will be seen from the accompanying table these figures suggest that amyloidosis is far more common in this hospital than in

other institutions from which statistics on this point are available. Fortunately, we are able to compare these figures with those obtained from the records of the Presbyterian Hospital where during the past seven years only six cases of amyloidosis have been observed in approximately eight hundred and fifty autopsies, an incidence of 0.7 per cent.

Also at the College of Physicians and Surgeons, where autopsy material is received from various hospitals, only twelve cases of amyloidosis have been seen in nearly one thousand autopsies. Statistics from foreign hospitals show, too, a far lower incidence. It has seemed to us that a consideration of the possible causes for this striking difference in frequency should prove of interest.

TABLE

	Chronic Tuberculosis	Cases Showing Amyloidosis	Incidence Per Cent.	Non-Tuberculous Cases	Cases Showing Amyloidosis	Incidence Per Cent.	Total Autopsies	Total Number Cases of Amyloidosis	Incidence Per Cent.
Montefiore Hospital.....	21	8	38.1	34	1	3.0	55	9	16.4
Presbyterian Hospital ..	50	2	4.0	800	4	0.5	850	6	0.7
College of Physicians and Surgeons	158	9	5.7	842	3	0.35	1,000	12	1.2
Vienna (Blum's statistics	2,500	221	8.8	15,653	76	0.49	18,153	297	1.5
Kiel (Wicht's statistics).							2700	97	3.6

It should be stated in the beginning that the number of autopsies in the Montefiore series (fifty-five) is rather small for any conclusive statistical study, but the fact that only sixteen[1] cases of amyloidosis have been found in one thousand, eight hundred and fifty autopsies outside of Montefiore Hospital goes far to prove that the high incidence there cannot be regarded as accidental.

In discussing this problem with Dr. Wachsmann, Superintendent of Montefiore Hospital, and others, it has been suggested that the patients in that institution differ from those in other general hospitals in at least three respects: (1) Most of the patients are foreign born. This was true of all of the cases of amyloidosis;

[1] Eighteen cases are recorded in the table but two of these were Montefiore cases, the material from which was sent to the College of Physicians and Surgeons.

(2) All patients belong to the Jewish race; (3) The tuberculous patients admitted differ in certain respects from those in many other hospitals.

Regarding the first factor, namely, the nationality of the patient, it has been stated by many who have attended autopsies in Berlin and Vienna, that amyloidosis is much more common in Europe than in America. The statistics from Vienna and Kiel (see table) seem to disprove this idea, since the percentage of amyloidosis in total number of autopsies as well as in cases where death was due to tuberculosis is not much higher than at the College of Physicians and Surgeons, and not nearly so high as in the Montefiore series.

At Vienna in calculating the percentage of tuberculous cases showing amyloid deposits, cases of acute general miliary tuberculosis and tuberculous pneumonia were probably included, whereas such cases were carefully omitted in the preparation of our statistics. We made this selection of tuberculous cases, firstly, because amyloid is practically never found in acute tuberculosis; and, secondly, because all the cases at Montefiore Hospital were chronic. Therefore a fair comparison necessitated a selection of cases of the same type at the other institutions. If we should include in our statistics from the College of Physicians and Surgeons and the Presbyterian Hospital deaths from acute tuberculosis the percentages of amyloidosis in tuberculosis would fall considerably lower than those for Vienna and Kiel.

Looking into the question of nationality of the six cases of amyloidosis at the Presbyterian Hospital, we find one Italian, one Englishman, two Hungarians, one American of German parentage, and one American negro. The percentage of foreign-born patients admitted to the hospital is about 40 per cent., but if to this group are added those of foreign parentage the percentage of "foreign" patients would probably reach 75. It is seen therefore that these figures do not give much support to the idea that foreigners are very much more susceptible to amyloid disease.

The second suggestion, that of a racial predisposition, is one upon which our available statistics are not altogether conclusive. At the College of Physicians and Surgeons no record of race has been kept. At the Presbyterian Hospital, although about 12 per cent. of the admissions are Hebrews, autopsies upon people of

Jewish faith are rare, comprising less than 5 per cent. of the total number. Thus we are able to compare the records of a hospital where all are Hebrews with those of another where there is only a small percentage of such cases. This comparison (see table), which shows at Montefiore Hospital an incidence of amyloidosis nine times as great in cases of chronic tuberculosis and six times as great in non-tuberculous cases as at the Presbyterian Hospital, certainly suggests a definite racial susceptibility. However we hesitate to form any conclusion on this point because a careful study of the cases at the two institutions, which brings us to the third possible predisposing factor, shows that the types of patients, especially those of chronic tuberculosis, admitted to the two institutions differ very materially.

The patients coming to autopsy from the tuberculosis division of Montefiore Hospital show as a rule far advanced pulmonary tuberculosis of long duration (average of those coming to autopsy, three years and four months). At the Presbyterian Hospital the cases of chronic pulmonary tuberculosis give a history usually of short duration (average of those coming to autopsy, nine months). While we have included in the fifty Presbyterian Hospital cases only those which were obviously chronic with cavity formation in lungs, or extensive bone lesions, we do not think that the comparison between these and the Montefiore cases is altogether satisfactory.

In order to settle conclusively the question of a racial susceptibility it will be necessary to obtain statistics from an institution receiving non-Jewish patients, more nearly of the type found at Montefiore Hospital.

The experimental work of Davidsohn and others, who have shown that certain strains of mice and rabbits are much more susceptible than others to the development of amyloidosis, is at least suggestive in this connection.

The tentative conclusions from this brief review of twenty-seven cases of amyloidosis seen in New York among nineteen hundred and five autopsies are as follows:

1. The influence of nationality upon the incidence of amy-

loidosis is negligible. That the condition is less common in America than in Europe is doubtful.

2. A predisposition to amyloid disease on the part of Hebrews is suggested, though not proved, by the high incidence observed at Montefiore Hospital.

3. The most important factor in determining the incidence of amyloidosis in any hospital is the type of patient admitted. Tuberculosis of long standing, involving extensive tissue destruction, is the condition most often associated with amyloidosis (70 per cent. in our cases).

(*b*) REPORT OF CASE WITH AMYLOID-LIKE DEPOSITS IN ORGANS

In eight of the nine cases of amyloidosis observed at Montefiore Hospital, and in all of the eighteen cases at the Presbyterian Hospital and the College of Physicians and Surgeons, the amyloid-containing organs showed the usual reaction with iodine. In one of the Montefiore cases, however, the reaction was entirely negative.

The case was that of a young man, twenty years old, who had shown symptoms of pulmonary tuberculosis for two years: cough, expectoration, hemoptysis, afternoon fever, etc.

At *autopsy* lungs showed extensive tuberculous lesions with numerous cavities in right lung. Spleen weighed 800 grams, was hard and on section presented the picture of a typical "sago" organ. The liver weighed 2,200 grams, was very firm and friable and showed the characteristic bacon-like translucency. In the kidneys no striking gross changes were apparent. The adrenals were rigid as though preserved in formalin.

Microscopical Examination: In the spleen, liver, adrenals and glomeruli of kidneys an extensive deposition of a hyaline pink-staining material was found, which presented the distribution characteristic of amyloid.

Application of iodine to the gross specimen, as stated above, gave no reaction. Negative results were likewise obtained with methyl-green and iodine-green. Methyl-violet gave in places a very faint reddish tinge which appeared to be more pronounced in the hyaline material in immediate proximity to the blood vessels. This faint staining was clearly seen in the liver sections. The

tests were applied on fresh tissue and on specimens fixed for a short time in formalin. Some of the sections were first treated with weak acetic acid in order to neutralize any alkali present.

There seems little doubt, therefore, that we are concerned here either with deposits of an incomplete amyloid material or a general hyalinosis, a condition not recognized by those who have worked in this field. It has been clearly demonstrated by Davidsohn and others in the experimental amyloidosis of mice and rabbits that there are at least two stages in the development of amyloid. In the earlier stage the deposits do not give the usual reaction. The reaction with the methyl-violet group of dyes appears first, usually in the spleen. The iodine reaction is obtained later. Only the completely finished product, which is found rarely in organs other than the spleen and liver, gives the iodine-sulphuric acid reaction. In the present case such an extensive deposition would suggest a late rather than an early stage. It is possible, however, that there may have been a very rapid formation and deposition of the incomplete amyloid during the last few weeks of the patient's life, and that sufficient time had not elapsed for its complete development. So far as we have been able to find out no case like this one, with such extensive deposits giving none of the usual reactions, has been described.

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REPORT ON TWO FORMS OF CARDIAC ABNORMALITY

HENRY A. RILEY, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons and the New York Nursery and Child's Hospital)

The study of the two hearts which form the basis of this report is presented more with the idea of placing the results on record than of giving in connection with it any extended discussion of the anatomical or pathological features of the organs. The

material was obtained from the Pathological Laboratory of the New York Nursery and Child's Hospital. During the past year we have met with almost all the common types of cardiac abnormality and in addition we have found three conditions of more than usual interest:

(*a*) Complete transposition of the arterial trunks.
(*b*) Complete absence of the interventricular septum.
(*c*) Aortic atresia and rudimentary left ventricle.

The first two will be shown at this meeting; the third will be made the subject of a separate report at some later date.

COMPLETE TRANSPOSITION OF THE ARTERIAL TRUNKS

CASE I. *Pathology.*—In this case, we find the ventricular portion of the heart to be normal in all its features; position, size, and composition of its costo-chondral surface. The heart is normally divided by its interventricular groove. The right ventricle externally appears somewhat larger than the left and forms the apex of the heart, but upon opening the organ, we find that the cavity of the left ventricle is larger than that of the right. The walls of the right ventricle are thicker than those of the left. The auriculo-ventricular valves are thin, delicate, and present extra flaps; the right auriculo-ventricular orifice being guarded by a valve which presents four flaps, the left auriculo-ventricular orifice by a valve with three flaps. The auricles are normally formed and separated from one another and there are no defects in the interauricular septum. The foramen ovale is still patent along its anterior border.

The great vessels present a condition of complete transposition, the aorta arising from the right ventricle, the pulmonary artery from the left. They emerge from the heart in an almost directly transverse plane close together and near the anterior aspect of the heart. They both pass upward and somewhat backward. The aorta then sweeps to the left and posteriorly over the bifurcation of the pulmonary artery, giving off its three great vessels from its superior aspect. The inferior aspect of the vessel receives the ductus arteriosus which is comparatively long, twisted,

and patent. The aorta then passes over the root of the left lung. The pulmonary artery passes upward and backward under cover of the aorta and divides into three vessels, the ductus arteriosus,

Photographs of reconstructions of the cardiac apparatus in three cat embryos—7, 9, 11 mm.—in length, showing the effect of torsion on the truncus arteriosus and the division of that vessel into the aorta and the pulmonary artery.

FIG. 1. 7 mm. cat embryo. *a*, right auricle; *b*, left auricle; *c*, common arterial trunk; *d*, interventricular septum; *e*, right ventricle; *f*, left ventricle.

which arises slightly to the left of the bifurcation, and the two pulmonary arteries. The coronary arteries are derived from the

aorta. The semilunar valves are normal both in number and arrangement as are also the pulmonary veins.

In this case we have an arrest of torsion of the great vessels whereby the orifice of the aorta remains on the right side, that of the pulmonary artery on the left. The interventricular septum,

FIG. 2. 9 mm. cat embryo. *c*, common arterial trunk. The truncus shows here the effect of the torsion.

rising from below and coming in contact with the septum trunci, establishes connection between the aorta and the right ventricle, and the pulmonary artery and the left ventricle. The difficult thing to reconcile with this course of events is that in this case

the ventricles have obeyed the tendency to rotate and have taken up their normal position, whereas the truncus which is supposed to obey the same factors in its rotation has completely ignored these factors and has remained unrotated.

Transposition of the Arterial Trunks.—According to the teachings of Rokitansky, transposition of the great vessels, *Rechtslage* of the aorta, as well as certain types of developmental stenosis of the aorta and pulmonary artery, are due to irregularities in the formation of the septum within the aortic bulb and to its malunion with the interventricular septum.

Types.—(*a*) Complete transposition in which the vessels arise from the ventricle to which they do not normally belong.

(*b*) Corrected transposition in which the vessels arise from the proper ventricle but present an inverted relationship to each other.

(*c*) Partial transposition.

In studying these conditions it is necessary for us to keep in mind the processes through which the heart passes in its development in order that we may understand the anomalies which arise through arrest of development or perverted development. In order that these factors may be clear in our minds, it may be well for us to review the steps in the development of these structures.

As will be remembered, the primitive heart tube in its growth becomes twisted upon itself in the shape of the letter S, the venous end being situated posteriorly and to the left in the position where the auricles will develop; in front of the venous end of the heart tube lies the ventricular portion and passing upward mesially and cephalad from the ventricular portion lies the common arterial trunk. There are two fixed points to be considered in order that we may understand the process of torsion which is to determine the picture in the truncus; the first, where the venous end of the heart enters the pericardial sac; the second, where the arterial trunks leave the pericardial sac. Continued growth within the pericardial sac along the direction already initiated by the S-shaped tube produces a torsion of 180 degrees of the common truncus by which the relationship of the adult aorta and the pulmonary artery is produced; this torsion is from the left to the right. By the time that this torsion has begun to make itself felt, the process of separation between the aorta and pulmonary artery has been initiated. This separation is produced by the appearance within the cavity of the truncus of two sets of ridges which according to their relationship with the ventricle, are called proximal and distal. The proximal set of two may be called ridges *A* and *B*, and a distal set of four, ridges 1, 2, 3 and 4. The result of the external forces of rotation or torsion on these ridges is the assumption by them of a spiral twist as they pass toward the heart along the interior of the tube. This spiral twist takes a clockwise direction from above downward and to the right; on the exterior of the tube can be found furrows which correspond to the internal ridges.

We will now follow in some detail the origin of the two sets of ridges

and their behavior in dividing the tube into the two large vessels. The ridges arise on sides of the tube opposite one another; the ridges *A* and *B* in the lower half, the ridges 1, 2, 3 and 4 in the upper half, of the truncus. They are so arranged that as they spiral downward in a clockwise direction one ridge of the proximal set meets and fuses with one ridge of the distal set

FIG. 3. 11 mm. cat embryo. *g*, aorta; *k*, pulmonary artery; *i*, ductus arteriosus. Complete separation of the truncus into the aorta and the pulmonary artery.

on either side of the tube. We have thus in the composition of each ridge three parts; one horn of the septum aorto-pulmonalis (will be described in detail later); one of the ridges 1 and 3 and one of the ridges *A* and *B*. Ridges 1 and 3 are large, 2 and 4 are small.

Prior to this there has been a separation of the fourth and sixth aortic arches external to the pericardial sac into what will later become the adult aorta and pulmonary artery. This so-called septum aorto-pulmonalis now invades the portion of the truncus which lies within the pericardial sac and adds itself to the factors producing a separation between the two vessels. We now have the two sets of ridges, *A* and *B*, and 1, 2, 3 and 4, and the septum aorto-pulmonalis. This septum pushes forward its two horns which thus enclose between them a free sickle-shaped edge and finally effects a junction with ridges 1 and 3, which in turn becomes continuous with ridges *A* and *B*. The process thus initiated progresses slowly, the sickle-shaped free margin passes caudally. The ridges become thicker and thicker until they fuse from above downward and the two vessels become completely separated. Through the spiral arrangement of the ridges we now have two curving tubes which looked at from above present a pulmonary artery passing forward and to the right, and an aorta passing posteriorly and to the left closely applied and wrapped about one another.

At a somewhat later time the vessels become independent through their separation by the external furrows which corresponded to the internal ridges. Having now seen the single trunk divided into two separate vessels, we can follow the development of the semilunar valves. Through the fusion of ridges 1 and 3 each vessel contains one half of each fused ridge and in addition the smaller ridge which lies between the larger ridges. The aorta contains ridge 2, the pulmonary artery ridge 4; these smaller ridges fade away distally but in the region of the junction with the ventricle they enlarge and become hollowed out, forming one of the semilunar valves; the other two valves are formed through similar processes taking place in the halves of ridges 1 and 3 left in each vessel.

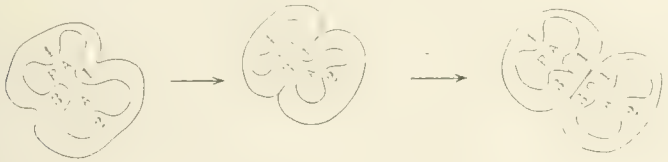
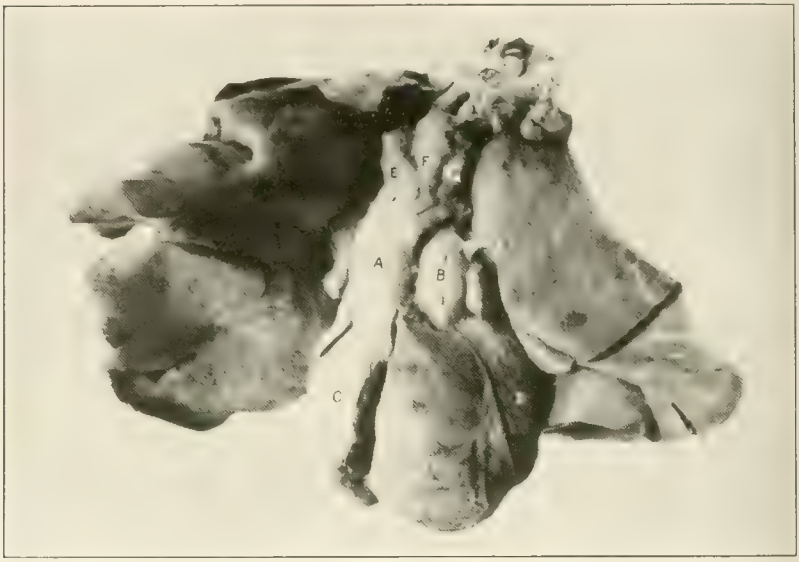


Diagram to illustrate the formation of the semilunar valves which develop by the hollowing out of the lowermost extremities of the ridges 1, 2, 3 and 4. The diagram also illustrates how ridges 1 and 3 participate in the formation of valve-flaps in both the aorta and pulmonary artery.

The two vessels now open into the ventricle which has become divided by the interventricular septum into the smaller right and the larger left half. This septum grows up from the floor and both anterior and posterior walls at the same time so that it presents two horns, an anterior and a posterior, containing between them a free sickle-shaped border. Meanwhile, the most inferior portions of ridges *A* and *B* have grown forward, joined, and have produced what we call the proximal bulb septum. Viewing this from above, we find that it passes from the right superiorly and posteriorly toward the

left anteriorly and inferiorly, and thus sets itself in a warped fashion on the interventricular septum which is growing upward to fuse with it.

The anterior cornu of the septum trunci fuses with the anterior horn of the interventricular septum, while the posterior cornu of the septum trunci spreads out on the posterior ventricular wall and fuses with the right extremity of the posterior endocardial cushion of the atrio-ventricular canal. If we follow the boundaries of the interventricular foramen, we now find them as follows: starting from above and behind we have the warped under surface of the septum trunci leading to the anterior cornu of the interventricular septum, advancing downward along the anterior wall to the inferior sickle-shaped intercornual margin, back and finally upward along the poste-



Photographs of the specimens embodied in the report.

FIG. 4. Complete transposition of the arterial trunks. *a*, aorta; *b*, pulmonary artery; *c*, right ventricle; *d*, left ventricle; *e*, innominate artery; *f*, carotid artery left; *g*, left subclavian.

rior cornu of the septum to the right extremity of the posterior endocardial cushion. This space is gradually closed in by downgrowth of the septum trunci and upgrowth of the interventricular septum until they meet and fuse.

As a result of the direction of the septum trunci toward the right end of the posterior endocardial cushion, we find the right atrio-ventricular opening close to the septum, the left separated from it by some distance.

Having seen the mechanism by which the great vessels become separated from one another, it is a comparatively easy matter to see how readily anoma-

lies of the arrangement of the great vessels may occur. Rokitansky, in his schema of the rotation of the great vessels, distinguishes two main groups according to whether the transposition was corrected or not with eight subdivisions in each group, the subdivisions being stages in the spiral twist and the form that the transposition would take in each subdivision were the spiral arrested at that point. In his schema *A* we find the transposition is corrected, for, in spite of the altered relation between the two great trunks, they are connected with the proper ventricle by a compensatory twist of the interventricular septum. In schema *B* we find, however, a true transposition of the trunks, there having been no correction and the trunks being connected with the wrong ventricle. The behavior of the interventricular septum determines whether correction takes place or not.

Partial Transposition.—This is relatively infrequent, but it exists and results in the large vessels arising from the same or a common ventricle and is due to the application of the interventricular septum to either one side or the other of the large vessels instead of between them. In such cases it is the rule to find the orifice of one or the other of the vessels encroached upon.

An anomalous septum may cut off an abnormal cavity as an origin for the two vessels. Unless some other anomaly is present and persists, complete transposition is incompatible with life, for we have the lesser circulation cut off and transformed into a closed circulation. This deprives the blood of any opportunity to gain access to the greater circulation unless it is by some abnormal channel as a patent ductus, a patent foramen ovale or a defect in the interventricular septum; correspondingly, the blood in the greater circulation fails to gain access to the lungs.

Defects of the Interventricular Septum.—As is usually the case with cardiac lesions, we often find other lesions associated with defects in the interventricular septum, the great vessels being often affected. This is readily understandable when we consider the close relationship between the two sets of structures.

Types: (*a*) Complete.
(*b*) Incomplete.

If complete we may find either a condition known as cor triloculare biatriatum or cor biloculare, depending upon the presence or absence of the interauricular septum. In association with the latter we may find a common auriculo-ventricular orifice and an undivided truncus.

These defects may be viewed mainly as arrests of development and in the majority of cases may be explained by a simple schema of the development of the interventricular septum. The septum develops from four component parts:

(*a*) The proximal septum trunci.
(*b*) The anterior horn of the interventricular septum.
(*c*) The posterior horn of the interventricular septum.
(*d*) The intercornual septum.

(*a*) Defects in the septum due to faulty development of the septum trunci constitute the most frequent type. The defect is located close under the orifices of the great vessels and occupies the area known as the "undefended space" or the pars membranacea.

(*b*) Defects due to the failure to develop of the anterior horn of the interventricular septum are located in front of the pars membranacea.

(*c*) Defects due to failure to develop in the posterior horn are correspondingly located behind the pars membranacea.

FIG. 5. Complete transposition of the arterial trunks. *a*, aorta; *b*, pulmonary artery; *c*, right ventricle; *d*, left ventricle; *e*, innominate artery; *f*, left carotid artery; *g*, left subclavian; *k*, ductus arteriosus; *i*, thymus; *l*, right auricle; *m*, left auricle.

(*d*) Defects due to faulty development of the intercornual portion may lie at any point in the septum, but they are more usually close up under the pars membranacea and are due to failure of the two septa to join.

COMPLETE ABSENCE OF THE INTERVENTRICULAR SEPTUM

Case II. *Pathology.*—Externally the heart is quite normal in shape. It weighs 170 grams, measures 8 × 7.5 × 6 cm., and has a fairly well defined and formed apex. There is, however, no interventricular groove to be seen and no indication of any division into two ventricles. The great vessels appear to arise from the heart on almost the same plane; the aorta from the right side,

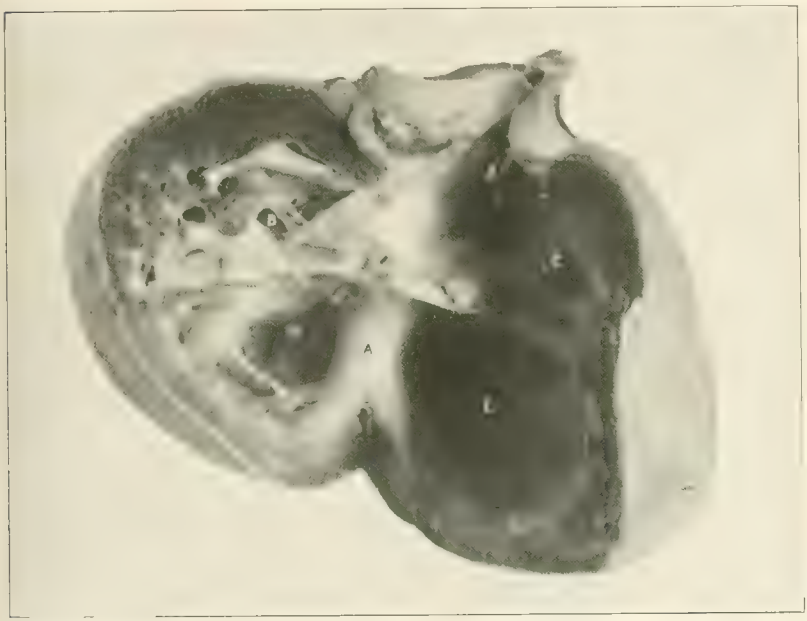


Fig. 6. Cor triloculare biatriatum. *a*, septum trunci; *b*, left auricle; *c*, right auricle; *d*, left ventricular portion of common ventricle; *e*, right ventricular portion of common ventricle.

the pulmonary artery from the left and slightly posterior so that we have here also a case of transposition of the vessels. The vessels are cut off so close to the heart that their further course cannot be followed but so far as we can see there is no indication of any ductus. Upon opening the auricles, we find that the right auricle is the larger, the right auriculo-ventricular orifice measuring 5 cm. in circumference; the left auricle is smaller, its auriculo-

ventricular orifice being 3 cm. in circumference. A rough estimate of the capacities of the two auricles would make that of the right three times that of the left. The right valve presents three flaps and several thickenings varying from 1 to 6 mm. in size. The left valve presents two main flaps which are broken up into a number of smaller ones by the insertion of anomalous chordæ tendineæ. The interauricular septum forms a common site of origin for both valves and a common papillary muscle receives chordæ from each of these valves.

There is but a single ventricle without any evidence of attempt at division in two by an interventricular septum. The walls are thick and heavy and present large papillary muscles and columnæ carneæ. The auriculo-ventricular orifices present at the posterior part of the ventricle, the orifices of the great vessels at the anterior part of the ventricle, the aortic on the right, the pulmonary on the left. These orifices are separated by a thick, heavy band of muscle fibers passing anteriorly and posteriorly between them and represent the proximal septum trunci.

I am much indebted to the anatomical department of the College of Physicians and Surgeons for permission to reproduce the reconstructions indicating the divisions of the truncus into the aorta and the pulmonary artery.

ADDITIONAL CASE REPORT

Since the report of the preceding case of complete transposition of the arterial trunks, it has been our good fortune to find another instance of the same pathological condition. The two hearts are almost identical and present pictures that agree in every essential point. In the second instance the child lived for about six hours after birth. There was a great deal of cyanosis affecting particularly the extremities, the hands and feet being a deep indigo.

Upon opening the thorax the organs were found to be intensely engorged and congested, the lungs being almost black. As soon as the pericardium was opened, the transposition was recognized and the organs were removed *in toto*. As in the previous case the aorta arises from the right ventricle, passes upward, giving off the great vessels from its convexity and receiving on its concavity the patent ductus arteriosus as it passes over the pulmonary artery. The pulmonary artery shows the same arrangement as we have seen previously, dividing into its two pulmonary arteries under cover of the aortic arch, the left one in turn giving off immediately the ductus arteriosus.

Upon opening the right auricle, it is found to be large and dilated; the

foramen ovale is patent along its anterior edge. The left auricle is considerably smaller than the right. The right ventricle presents the origin of the aorta. Passing posteriorly and slightly to the right behind the origin of the aorta is a thick heavy band of muscle fibers which represents the lower margin of the primitive septum trunci. It serves to separate the aorta and the right auricle and from its posterior aspect it gives origin to one of the flaps of the tricuspid valve. The origin of the two coronary arteries can be seen in the posterior and left sinuses of Valsalva of the aorta. The flaps of the mitral and tricuspid valves are normal in structure and number. The left ventricle presents the origin of the pulmonary artery arising anteriorly and close to the interventricular septum. The interventricular septum presents no defects.

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Discussion:

Dr. MacCallum: I think Dr. Riley has covered the ground pretty thoroughly. There is one striking point in this connection and that is the mathematical regularity with which certain principles are carried out in transpositions of the great vessels. In making out his schema Rokitansky was able to prophesy anomalies of the great vessels and several types which had not been reported prior to this, but which he had figured as possible in his schema were later reported by different observers. A good deal of light can be thrown on this subject by cutting the hearts transversely close under the great vessels to show the disposition of the aortic and interventricular septa and by comparing them with pathological hearts cut in a similar fashion. A better idea of the relationships can be gathered in this fashion than by cutting the heart in the usual fashion through the valve flaps.

Dr. Larkin: I have had rather an interesting anomaly of the heart recently. The child had spina bifida. In these cases anomalies of the cardia are not infrequent. The child presented several abnormal conditions. This abnormality of the heart has never before been described. The charts of Dr. Riley give an idea of the disposition of the vessels. In my case the aorta leads directly into the right and also into the left ventricle, the pulmonary artery being normal. The aorta is normal until the base of the heart, where there is a lack of the septum. The aorta is bifurcating, one part goes to the right ventricle, one to the left.

Dr. Riley: The explanation is I think that the interventricular septum comes up from below and the septum trunci comes from above; they do not meet and the interventricular septum separates the aorta into two parts.

Dr. Larkin: There is no evidence of any upper septum at all.

Dr. Riley: You have a division of the truncus into two vessels.

Dr. Larkin: The pulmonary goes into the right ventricle, the aorta into the left. There is no abnormality of the pulmonary.

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SOME ATTEMPTS TO PRODUCE EXPERIMENTALLY CONDITIONS OF SYMPATHETICOTONUS, VAGOTONUS, AND HYPERTHYROIDISM[1]

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York

By Dr. ABRAHAM TROELL, Stockholm, Sweden
Docent of Surgery

IN the past few years, the question of the vegetative nervous system and the ductless glands has been the subject of unusually active study. More and more the important part played by these sets of organs, in physiology as well as in pathology, has been properly appreciated, and those investigators concerned particularly in clinical work have felt that there were strong reasons for considering these organs as a whole, or as important component parts of a whole, in which the regular function of every subsidiary part was extremely valuable to the health of the entire organism, while disorders might lead to a pathological condition. Abnormalities at one point might disturb the equilibrium normally existing between the sympathetic and vagus, or between various groups of hormone-forming organs. Clinically such a condition would reveal itself either as an *eo ipso* more or less distinct disease (such as exophthalmic goiter), or merely in the form of less striking symptoms, which, on account of other simultaneous symptoms, are referred to entirely different groups (such as cholelithiasis).[2] In view of the very great difficulties often encountered

[1] Received for publication, July 6, 1915.

[2] With regard to the nomenclature there is not yet full agreement. Some distinguish as subdivisions of the *vegetative nervous system* (i.e. the system that, independently of the cerebrospinal nervous system, supplies the smooth muscles, glands, etc.): (1) the vagus (the autonomic system), and (2) the sympathetic (the sympathetic system). Some have assigned—and the advantage of this is questionable—to the whole the term vegetative or autonomic system, which latter they divided into (1) craniobulbar and sacral portion (including the vagus), and (2), a thoracic part (sympathetic). By hormones (Bayliss, Starling) are meant the chemical products secreted by the ductless glands in their regular metabolic function. See Eppinger and Hess. (Ztschr. f. klin. Med. 1909, lxvii 345; 1909, lxviii, 205); K. Petren and Thorling (Ztschr. f. klin. Med., 1911, lxxiii, 27); Timme (J. Am. M. Ass., 1915, lxiv, 321; J. Nerv. & Mental Dis., 1914, xli, 745); Thies (Mitteil. a. d. Grenzgeb. der Med. u. Chir., xxvii, 1914, 389; 1915, xxviii, 415) Biedl (Innere Secretion, 1913, i, 210) and others.

in a proper interpretation of clinical observations, it is natural that attempts have been made to study the effect of the removal of one of the glands in question, for example, the thyroid, or of supplying the organism with a hormone or with some substance of like effect, such as adrenalin. This substance was chosen with a view as to its affinity either for the sympathetic or for the autonomic nerve system, that is, as to whether it was sympatheticotonic or vagotonic. It has thus been possible to a certain extent to study either the sympatheticotonic or vagotonic symptoms of diseases.

There is, however, another possible mode of producing sympatheticotonic or vagotonic conditions, at least in theory. It is based on experiments, made chiefly during a period of several years, by Langley,[1] in order to determine the possibility of an anatomical and functional union of nerves of different kinds, by resorting to suture. Flourens[2], Forssman,[3] Ballance,[4] and others, proved that the central end of one nerve might make functional union with the peripheral end of another nerve of similar kind, for instance, the accessorius with the facialis. Langley and Anderson[5] showed, by systematic combinations covering practically every possible variation, that such a union between the central end of one nerve and the peripheral end of another, regardless of the character of the nerves, was nearly always a possibility.

In this series of experiments, Langley and Anderson performed the following operation on a cat. The phrenic and sympathetic on

[1] Langley's (J. of Physiol., 1904, xxxi, 365) object was chiefly to obtain "functionally united" nerve-ends, to study the physiological aspect, in other words, and not to touch upon the histological process. He merely wanted to get two nerve-ends to grow together after suture, in such a manner that the new (mixed) nerve becomes a functioning whole replacing the original nerve communication. He regards as of minor importance, in this connection, the question as to how the nerve-ends grow together: whether the fibers of both the proximal and distal remnants coöperate actively (the coalescence theory) or whether only the axial cylinders of the former expand peripherally (the outgrowth theory). Langley's investigations were carried on before the revealing of the altogether new outlook and possibilities of progress that resulted from our having succeeded in cultivating tissues *in vitro* and in observing directly and in detail the manner in which living cells grow and are regenerated. On this subject, see Harrison (Neue Versuche u. Beob. über. d. Entwicklung der periph. Nerven der Wirbeltiere, Bonn, 1904; Anat. Rec., 1907, vii, 116); Lewis and Lewis (Anat. Rec v, p. 227; vi, p. 7, 195, 207; J. Exp. Med. 1912, xv, 397; xvi, 421, 1913; xviii, 412); recapitulatory articles by Cushing (Johns Hopkins Hosp. Bull. 1908, xvi, 77; 1910, xxi, 325) and F Henschen. (Hygiea. 1914, lxxvi, 1101)

[2] Ann. d. Sc. nat., 1828, iii, 113. (cit. J. Physiol., 1904, xxxi, 365.)

[3] Ziegler's Beitr. z. path. Anat., 1898, xxiv, 56.

[4] Lancet, Lond., 1902, i, 379, 591.

[5] J. of Physiol., 1897, xxii, 215; 1898, xxiii, 240; 1904, xxx, 439; 1904, xxxi, 365, 418

the left side of the animal's neck were cut. The proximal end of the former nerve was sewn to the distal end of the latter, and the proximal end of the latter to the distal end of the former. On the right side, the lower half of the cervical sympathetic was excised. The visible consequences of these steps were: equal paralytic effects on the two sides of the head for about two months (in the eye a constriction of the pupil, etc.). These changes then gradually diminished on the left side. After 197 days, still a difference was noticeable between the paralytic symptoms on both sides, and while electrical stimulation of the left cervical sympathetic immediately below the superior ganglion produced the customary effect in the corresponding eye (dilatation of the pupil, etc.), nothing resulted from a stimulation of the right cervical sympathetic. Similar experiments were made on two other cats. Langley and Anderson point out *en passant* that at no time was any change observable in the nictitating membrane, eyelids or pupil, to correspond with respiration.

The last mentioned method of experiment may be of interest in quite another connection than that which Langley and Anderson had in mind. Under normal conditions the phrenic nerve is the path over which automatic impulses constantly pass to the diaphragm. Therefore, if it were possible to transmit and, as it were, accumulate to the sympathetic nervous system, for any length of time, the stimuli which are conducted from the central nervous system to the periphery through this nerve, it might be possible also in this way through the mediation of a sympatheticotonic condition to produce changes in the organism similar to the diseased conditions that may be conjectured to be partly due to mental emotions. At any rate there would be sufficient ground for ascertaining whether an anastomosis experimentally produced and of some duration, between the central end of the phrenic and some point of the sympathetic or vagus, could result in general alterations of the organism.

A short preliminary report of an investigation of this nature has just been published by

Cannon, Binger, and Fitz.[1] They formulate the object of their investigations as follows: "Interest in the bodily changes during or following emotional excitement led us to inquire concerning the nature of certain diseases often reported as having emotional origin. We proceeded on the theory that repeated emotional experiences might lower a naturally high neurone threshold and thus result in frequent stimulation of parts which normally are only occasionally roused to special activity." In these experiments the anterior root of the phrenic on a cat "was fused with" the right sympathetic cord of the neck. Thus, after regeneration, "there was delivered to neurones in the superior cervical ganglion a volley of impulses every time the animal breathed." The first results of these experiments were made known in a lecture by Cannon late in the autumn of 1914. At that time four animals had been under observation for about five months after the nerve operations, all of which appeared now to present more or less evidence of hyperthryoidism concerning which I shall speak later.

Cannon's report was undeniably of great interest. If, as he claimed, it was possible to obtain, through a "hyperstimulation" of one sympathetic brought about in the way described, the same symptoms that characterize Graves' disease in human beings, a very profitable mode of study and interpretation of this disease would thus be opened up.

At the time of Cannon's announcement, I was in Professor MacCallum's laboratory in New York (Department of Pathology, Columbia University), engaged in investigations in the same field.[2] I was, therefore, very glad to take advantage of the latter's suggestion to repeat Cannon's experiments with certain modifications — to unite in the neck the proximal end of the phrenic nerve with that portion of the sympathetic or vagus respectively that goes downward to the thorax. In order to escape, as far as the vagus is concerned, the disturbing influence on the heart of a possible direct chronic

[1] Am. J. Physiol., 1915, xxxvi, 303.

[2] Some Attempts to Produce Exophthalmos experimentally. (In press.)

irritation of the vagus fibers going to that organ, I considered it more advisable to perform the anastomosis of the nerves down in the thorax, right over the diaphragm. The technique would be somewhat complicated by this change, but it would involve the consequence of placing a greater portion of the sympathetic or autonomic nervous system in a condition of stimulation than was the case in Cannon's union of the phrenic with the part of the cervical sympathetic ascending into the head.

Therefore, I performed a number of operations on the neck, in which one phrenic and one sympathetic were severed and then sewed together, so that the proximal end of the former was connected with the distal end of the latter, immediately under or right at the superior cervical ganglion; phrenic impulses of central origin would then chiefly influence the smooth muscles of the orbit and eye. I performed a second series of operations in the thorax and, after cutting off the proper nerve-trunks, united the proximal end of the phrenic with the distal (directed toward the abdominal cavity) end of the sympathetic or vagus. A detailed report of these experiments is given at the end of this paper.

Besides the three cases discussed in the first series of cases which were operated upon on December 9, 1914, December 12, 1914, and March 5, 1915, respectively, five other animals (dogs, cats, rabbits) were similarly treated. But there would be no special interest in a statement of their cases here, as they died within three weeks after the operation, without having shown any other characteristic symptoms beyond the constriction of pupil, palpebral fissure, etc., which are regular signs of sympathetic paralysis. Causes of death were: narcotic intoxication, pneumonia, infection, postoperative hæmorrhage. About 3 cm. of the sympathetic were always excised before the nerve suture was made.

Of the 14 thoracic cases in the second series of cases, Nos. 1 to 6, 12, and 13 were operated on December 10 to 29, 1914, the others, January 2 to April 3, 1915. In addition operations were made on five animals who died within 1 to 10 days. The cause of death in all these cases was infection following the operation. In one case the infection started from the tracheotomy wound and was combined with general emphysema. All the animals were anæsthetized by intratracheal insufflation by the Meltzer-Auer method. The technique was not

entirely the same for dogs as for cats. In the case of the former, after general narcosis had been brought about in the regular way, intubation *per os* was easy with a half-rigid catheter. But with cats this method presented such great difficulties that I preferred carrying out a tracheotomy first and then inserting a tube through the tracheal wound. The tracheal wound was always closed after performing the nerve-anastomosis in the thorax. Never, except in the case described above, did it cause any complications. The thoracotomy was usually performed in the seventh or more commonly, the eighth interspace. After carefully stopping the hæmorrhage in the soft parts, a self-retaining retractor was inserted and its blades separated widely. The lower lobe of the lung was pushed up and kept away from the field of operation by a gauze compress. To make possible a union of the phrenic and sympathetic, it was often necessary in order to mobilize a sufficiently large section of the sympathetic, to cut one of the sympathetic roots. All nerve-sutures were made end-to-end with very fine silk on a fine needle. Before closing the thoracic cavity it was in some cases filled with a physiological salt solution so that the lung subsequently might regain its normal volume with less difficulty; but the general post-operative condition of the animals showed no resulting improvement from that modification. As a rule the animals recovered quickly and seemed quite well 3 or 4 days later.

Before taking up a discussion of the various details in the report of the experiments, it may be well to consider the length of time during which the animals were observed after operation, as well as the purely anatomical viewpoints of the question of regeneration at the sutured nerve-ends.

Although the time elapsing between the suturing of the nerves and the carrying out of the final investigations was never very long (the shortest period, 20 days, Case 3; the longest, 175 days, Case B), for any animal, it may still, on the whole, have been long enough to enable the nerve-communication to be restored. In one of his experiments on cats, Langley[1] found that after 112 days the proximal end of the phrenic and the distal end of the sympathetic, which had been sewed together on the neck, had united so completely, that electric stimulation of the nerve proximal to the point of suture produced the customary effect of sympathetic stimulation (dilatation of the pupil, constriction of arteries in the ear, etc.). A result similar in principle was

[1] J. Physiol., 1904, xxxi, 365.

obtained by him within a month after sewing together the vagus and sympathetic on the neck.[1]

With regard to the healing up at the nerve suture-place in my cases I noticed as a rule, a distinct macroscopic difference, depending on the age of the nerve anastomosis. In Cases 1, 2, 3, and 8 (84, 45, 20 and 70 days, respectively after operation), the place of suture made the impression of a callous-like swelling; in Cases A, B, 4, 6, 7, 9, 10, 11 (131, 175, 107, 100, 57, 27, 87, 64, days respectively after operation), it was smooth and slim and impossible to distinguish with the naked eye. But it must be noted that in the last three cases it was with the vagus that the phrenic had been sewed (cases 9, 10, and 11), thus suggesting that these more closely related nerves are more quickly healed than when phrenic and sympathetic are united.

The fact that nerves of similar or identical nature really have a very great tendency to heal together is shown beautifully by Cases 12 and 13; 99 days or 73 days respectively after one phrenic and both vagi, or, in the other case, one phrenic, both vagi and one sympathetic, had been severed, all these nerves had neatly healed except the phrenic in the former case and the sympathetic in the latter. Experimentally the same fact had been ascertained before (Langley[2] and others). The tendency to regeneration is so strong that, as Cushing[3] puts it, it is almost impossible to prevent some reunion of divided nerve-fibers with their original central ends, or with the central ends of fibers from other sources which were unavoidably cut through during the operation. This is all the more interesting since we have long been taught that such a reunion is not to be expected, unless the fibers of a severed trunk are encouraged to find their proper connection by a surgical approximation of the stumps with suture or by some other means.

The histological examination of the place of nerve suture, which I made in some cases, led to a corroboration of the impression I had obtained with the naked eye, that the healing up of the connected nerve ends was satisfactory. This microscopical study was made of Cases 1 and 3, as well as of another case not included in the tables, which resulted in death on the day following the sewing

[1] Cit. Timme, J. Nerv. Ment. Dis., 1914, xli, 762.
[2] J. Physiol., 1897, xxii, 215.
[3] Johns Hopkins Hosp. Bull., 1905, xvi, 77.

together of the phrenicus sinister with vagus, sinister. Bielschowsky's stain gave the best picture; poorer pictures resulted from Stroebe's method and from Weigert's iron hæmotoxylin stain.[1] Serial sections were not made and thus it was not possible to get any completely exact conception of conditions at the point of suture. Yet, on both sides of the latter were seen well stained axones which, in the phrenic and vagus, sometimes had medullary sheathes. As in Langley's observations[2] I also found that some of the axones had not grown over the place of suture and regenerated, but had lost their way in the connective-tissue growth and ended blindly.

I have still to discuss the data in the experiments as to the condition of the animals after the operations. As to this point, I have taken into consideration the changes in the general condition (weight, pulse, carbohydrate tolerance, etc.) and local symptoms (eye changes).

Have the results been of such a nature as to show, in the operated cases, a symptom-complex indicative of sympatheticotonic or vagotonic conditions, or even of hyperthyroidism? First it must be stated what symptoms in dogs and cats are characteristic of the pathological conditions referred to, particularly of hyperthyroidism. A precise statement of this matter is impossible.

Graves' disease is a very unusual disease in dogs. I went through the last two annual volumes accessible to me of Ellenberger's and Schütz's *Jahresbericht über die Leistungen* auf dem Gebiete *der Veterinärmedizin* without finding anything on this subject. Eggers and Maury[3] have related a case of Graves' disease in a five-week old dog. The thyroid was enlarged, and (as observed at the operation, profusely supplied with vessels; the pulse was rapid 160 to 170 per minute), a fine tremor was present, but no unmistakable exophthalmos. Diagnosis was made of Graves' disease, thyroidectomy was performed, a generally good condition ensued but was of short duration. Six weeks later the dog died suddenly without reappearance of the old symptoms. The morphological aspect of the thyroid gland was that of an exophthalmic goiter.

[1] In staining, Schmorl's description of the methods (Path. anat. Untersuchungsmethoden. Leipzig, 1907, p. 223) was followed.

[2] J. Physiol., 1897, xxii, 215.

[3] Struma simplex (common goiter) seems to be frequent in dogs as well as horses (Hutchinson, Mills). Gorig has reported a case of Graves' disease in a cow (Deutsch. Thierag., Karlsruhe, 1898, v, i, 306) Cit. Eggers and Maury (Ann. of Surg., Phila., 1907, October).

It is particularly difficult to judge the cases of *experimental Graves' disease* in animals, recorded in the literature. Baruch enumerated the following symptoms of Graves' disease in dogs, having observed the symptoms in a number of animals after intraperitoneal injection of freshly ground human goiter: irritability, nervousness, extreme emaciation, falling out of hair, diarrhœa, tachycardia, glycosuria, lymphocytosis and sometimes exophthalmos.[1] It is not surprising that the introduction into the animal organism of a rather large quantity of heterogeneous proteid material of thyroid nature all at one time, has the effect of a poison. Carlson[2] makes the interesting statement that the same effect can be produced in rabbits, rats, and guinea pigs by feeding them with desiccated muscular tissue as with desiccated thyroid preparation. Under these circumstances an increase in pulse rate, fever, general weakness, emaciation, etc., may assuredly arise without assuming that a typical case of Graves' disease has been produced. The most characteristic feature about all these experiments is that experiments made by one investigator with positive results seldom give the same result when performed by another. In the few cases in which unquestionable Graves' disease symptoms are declared to have been produced, they have never remained evident for long.

The recognition of *sympatheticotonic and vagotonic conditions* is just as vague, or even more so. Macleod[3] showed among other things, that stimulation of the splanchnic nerve produces hyperglycæmia (provided the adrenals are intact); he obtained the same effect, after severing the plexus hepaticus, by electrical stimulation of the peripheral cross-section. Pawlow, Schiff, Van Yzeren, Cannon, and others, observed that stimulation of the vagus resulted in increased activity of the stomach glands.[4] And Timme[5] ascertained a histological hyperplasia of the mucous membranes in the stomach resulting from a chronic stimulation of the vagus lasting several months (produced by applying gently a ligature around each vagus). Finally Cannon, Binger, and Fitz observed in their experiments on cats, mentioned above, the presence of a marked tachycardia; the average heart rate was 222 while in normal case 165. Besides there were loose movements of the bowels, falling out of the hair; the cats were unusually excitable. Alterations of basal metabolism also occurred; the average heat loss per kilo per 24 hours in normal adult cats was 44 calories, but in four of the experimental animals it was 66, and in one, 112. The latter died sooner than the others and at the autopsy the adrenals were found to have nearly thrice the average weight. Finally still another symptom was mentioned:

[1] Zentralbl. f. Chir., 1911, xxxviii, 1153; 1912, xxxix, 310. Also see Bircher, Zentralbl. f. Chir., 1912, xxxix, 138; Klose, Ergebn. d. innere. Med. u. Kinderh. von Kraus, 1913, p. 167; Biedl, Innere Sekretion, 1913, p. 210.

[2] Biedl. loc. cit.

[3] Diabetes; its Pathological Physiology, 1913.

[4] Timme, J. Nerv. & Ment. Dis., 1914, xli, 745.

[5] J. Nerv. & Ment. Dis., 1913, xl, 311.

dilatation of the pupil on the operated side in dim light, and in one case, respiratory hippus.

It is perhaps proper here to give the division of Graves' disease symptoms into sympatheticotonic and vagotonic, according to Eppinger. The immediate source of this table is Halsted.[1]

SYMPATHETICOTONIC GRAVES' DISEASE SYMPTOMS

1. Pronounced protrusion of the bulbs
2. Graefe absent
3. Loewi's sign positive
4. Möbius positive
5. Dry bulbs
6. Greatly increased activity of the heart with less pronounced subjective symptoms.
7. Sweating and diarrhœa absent
8. Falling out of hair
9. Eosinophilia absent
10. Inclination to fever
11. Alimentary glycosuria
12. Refractory behavior to pilocarpine.

VAGOTONIC GRAVES' DISEASE SYMPTOMS

1. Relatively moderate degree of tachycardia
2. Pronounced subjective heart symptoms
3. Graefe definite
4. Wide lid-clefts
5. Möbius absent
6. Slight protrusion of the bulbs
7. Increased lacrymation
8. Profuse sweating
9. Diarrhœa
10. Disturbances of digestion
11. Eosinophilia likely
12. Alimentary glycosuria absent
13. No adrenalin glycosuria
14. Pigmentation.

In stating the symptoms observed in the animals in my experiments, a distinction must be made between dogs and cats. Psychical peculiarities could not be observed in any of them (except dog No. 2, who seemed to be very sensitive after operation). But the physical observation was repeated almost throughout that the dogs became leaner and the cats fatter after the operation, no matter what was done to them.[2] Yet it must be noted that such was also the case in all the animals kept in the laboratory during the same winter. I observed it, among other things, in a score of dogs in which one or more spleen-vessels had been ligated, in

[1] Johns Hopkins Hosp. Bull. 1914, xxv, 224.

[2] Exceptions were the cats C and 11. The former became very lean and developed a constant deterioration in general condition. Yet there was a distinct explanation in the post-operative infection that led to death. The nerve suture was found at the autopsy to have broken. The other cat was quite well during the first 6 weeks following the operation, then, like the other animals, at that time living in the same room, she was struck by a severe infection, giving symptoms particularly from the respiratory organs.

dogs from which one sympathetic neck ganglion had been removed or in which Eck's fistula had been made, or which had not been operated on at all, and I observed it as well in cats in which one adrenal had been removed, etc. It seems quite likely that other circumstances than those directly connected with the operation caused the difference in the conditions of nourishment. The dogs supplied to the laboratory usually presented a rather good general condition on their arrival, while the cats, on the other hand, were starved and in a bad state. But conditions in the laboratory were such that the dogs had to be kept in a place more unfavorable as to temperature, etc., than were the cats.

Another difference observed was that the dogs began to lose their hair at most two months after being brought to the laboratory and to show signs of itching and scratching. But this phenomenon also was true of all the other dogs kept in the laboratory, operated or not operated on. The cause was probably mange, which I am informed by colleagues is a rather common thing in such laboratory animals.

The pulse rate was never very high. It was always taken at about the same hour in the morning, the hand being placed on the animal's heart and the beats being counted for a whole minute. The average rate for dogs was about 120—lowest 97, highest 133, 21 observations in all; for cats about 130—lowest 113, highest 141, 41 observations in all. Three cats had an average rate varying between 132 and 162 before the operation (6 counts) and 2 of these (Nos. 8 and 11) varied between 135 and 141 during the days immediately following operation (6 counts) and between 142 and 149 during a couple of days, two months after the operation (10 counts).

For purposes of comparison the following counts made on other dogs during the same period may be mentioned.

In a dog the upper half of whose spleen had been ligated a month ago, the heart rate had an average of 119 (3 counts), in another 108 (2 counts). The corresponding figure for a dog whose upper sympathetic neck ganglion had been gently ligated two

months before, was 102 (2 counts).[1] It was 112 (4 counts) for another dog, whose two ovaries, as well as the upper sympathetic neck ganglion had been removed three weeks earlier, and who at the time of the pulse counts was fed on a total of 8 gms. thyroid extract, in two days. A dog which had undergone ovariotomy on both sides six weeks previously and which during the period of the pulse counts received 2 to 5 gms. of thyroid extract daily had an average rate of 117 (16 counts). Finally, the figure was 146 (27 counts) for a dog whose ovaries and one sympathetic neck ganglion had been removed and then fed on 2 to 3 gms. thyroid extract daily for two weeks after which she received 25 gms. fresh human colloid goiter grafted intraperitoneally. After the graft the heart rate of this animal rose quickly and considerably and reached on one day 198 (the highest I have ever observed in any dog).[2]

The fluctuations in pulse rate in these animals coincided, yet with still great variation, with another change, that in the carbohydrate metabolism.

It is well known clinically, that patients with Graves' disease sometimes develop glycosuria. It has also been found that this symptom may be produced in animals or human beings by administering thyroid products (the dogs just mentioned, one of which I fed on thyroid extract and in the other of which was grafted human colloid goiter intraperitoneally, had during the time in question, sugar in their urine). Crowe and Wislocki[3] found that the removal of one adrenal, either one, causes temporary glycosuria. Macleod's observations of the same symptom after stimulation of the splanchnic nerve, has already been mentioned. Thus it is evident—surely could more examples be given—that the endocrine organs do have an influence on the metabolism of carbohydrates, an influence whose mode of action is extremely complicated and by no means plainly understood.

Aside from the qualitative test for sugar in the urine the simplest way of detecting possible irregularities in the metabolism is probably the determination of the limit of

[1] Another fact about the dog that may be of a certain interest in view of what is reported later in the paper, is that his tolerance for saccharose was under 5.

[2] Another fact worth noting about this dog was the changes in weight and loss of hair. As long as the animal was kept (in a separate cage) in the same room with the cats, the weight remained fairly constant and no loss of hair was observed. But when she was placed (about 3½ weeks later) in a somewhat colder place, together with all the other dogs, a pronounced emaciation set in, accompanied by loss of hair.

[3] Beitr. f. klin. Chir., 1914, xcv, 8.

assimilation of the organism for a certain kind of sugar. This limit is said to be reached when the sugar administered *per os* leaves it with the urine. In healthy human beings this limit — the sugar tolerance — is pretty high, though nevertheless subject to individual variations. Data on sugar tolerance in animals are found in the literature but differ considerably. According to Hoppe-Seyler[1] it amounts, for saccharose, in normal dogs, to 20 to 30 gms.; according to Goetsch, Cushing and Jacobson[2] to 10 gms. per kilo body weight, when sugar is introduced *per os*.

In my animals I tried to determine whether the nerve operations performed might have any influence on the sugar tolerance. A study of the experiments will show that no decisive indication is obtainable, either with regard to the place or the mode of the nerve anastomosis or to the length of the period elapsing after the operation.

For the sake of brevity, I have here given simple numerical data on the sugar tolerance. The fact that an animal weighing 1 kg. is able to consume 10 gms. pure saccharose, dissolved in water with a little milk, without the urine's giving a positive sugar reaction next day, I thus indicate by saying that its sugar tolerance is more than 10. But if the same animal, conditions otherwise remaining unchanged, after consuming 15 gms. of saccharose, shows sugar in the urine, I state this by saying that its sugar tolerance is between 15 and 10, etc. As reagents I used both Nylander's and Benedict's solutions in each test. An uncertain result with the latter I call negative (Benedict's reaction is possibly somewhat more sensitive than Nylander's).[3] Saccharose was chosen for the test, partly because, if the animals drooled into the cages after the administration of the sugar, there would be no danger of contamination of the urine by a reducing substance, a possible risk in the case of glucose administered by mouth. During the days set apart for the determination of the tolerance the animals were kept in metabolism cages.

Two months after performing the phrenico-sympathetic anastomosis on dogs, the assimilation limit for saccharose lay between 15 and 12.5, which is remarkably high, in Case 7; three or four months after operation it was considerably lower, below 10 in Cases 1 and A. And about three months after a phrenic vagus

[1] Virchow's Arch. f. path. Anat., 1880, v, 144.

[2] Bull. Johns Hopkins Hosp., 1911, xxii, 165.

[3] See Hawk's Physiological Chemistry, Philadelphia, 1912, p. 328; and Wood's Chemical and Microscopical Diagnosis, New York and London; 1912, p. 513.

anastomosis, in Case 10, it was as low as at about the same period after a simple phrenicotomy in Case 12; in both cases between 5 and 2.5. In cats the figures were invariably low. Yet it should not be ignored that, excepting Case 5, they were somewhat higher in tests made on animals not operated upon, than in those operated upon. In Cases 8 and 14 the sugar tolerance was between 10 and 5; in other words, above 5 before the operation. In Case 11, it was between 5 and 2.5 before operation; the same figure as was found in Case 5 three and a half months after phrenicosympathetic anastomosis; and the remaining three cats (Cases B, 4 and 6) 3½ to 6 months after operation, likewise with phrenicosympathetic anastomosis, displayed an assimilation limit between 2.5 and .63. Spontaneous glycosuria or albuminuria was found in spite of frequent examination in only one case and temporarily, No. 8: glycosuria during a couple of days after operation and never on any other occasion.

As far as dogs are concerned, I had but one opportunity to compare with the sugar tolerance in an animal on which no nerve anastomosis had been performed. This dog (already mentioned in this paper) had an assimilation limit below 5, two months after ligation of one of the superior sympathetic neck ganglia. My last investigations on cats (Cases 8, 11, 14) I planned in the following way. The sugar tolerance was tested in each case at about the same time. Then I performed in the thoracic cavity in one of the cats a phrenicosympathetic anastomosis and in another a phrenicovagus anastomosis, not operating on the third animal. Afterward I kept them all in the same cage and under quite equal conditions. They did very well for the first six weeks. Whereupon they were struck by an infection (catarrhal disease of the respiratory tract) and developed for a time a rather bad general condition as well as did all the other animals in this room. The two cats operated upon improved, as to a certain extent did the third animal, not operated on, which died after two months before I was able to carry out another assimilation test. Cat B showed the same sugar tolerance, 111 days after performing the nerve anastomosis as she did after 175 days.

As for the remaining symptoms indicative of a condition of sympatheticotonus, vagotonus, or hyperthyroidism, it may be briefly stated that no unquestionable ones were found. Loewi's adrenalin test was negative

in the five cases in which it was applied. In the eyes no other symptoms were found than the customary effects (paralysis) on the operated side. I was particularly looking for a possible appearance of the interesting phenomenon which Cannon observed in one of his animals but which Langley could not find; namely, exophthalmos and the respiratory hippus. In spite of numerous investigations, both in broad daylight and in dim light, I never could detect any such phenomenon either in dogs or in cats.

Equally unsatisfactory were the macroscopic and microscopic findings in the organs at autopsy. In dogs 1 (anastomosis of the right phrenicosympathetic) and 13 (sympatheticotomy on left side) there was found, in the abdomen, a very peculiar picture of a seemingly chronic inflammatory nature. Numerous firm adhesions and strands of connective tissue were present, the intestines showed occasional local saclike extensions and the mesenteric glands were enlarged, indurated, and pigmented.

I have no information regarding the real nature of these changes. In the rather numerous autopsies I have had occasion to perform on dogs, I have observed them in only one additional case, and that in a dog who had already undergone ovariotomy on both sides and, three weeks later, had been fed daily 2 to 5 gms. thyroid extract for eighteen days. Here glycosuria and a marked deterioration of the general condition developed. After stopping thyroid feeding, the glycosuria disappeared. The animal died a few days later of pneumonia, however. The autopsy showed, among other things, exactly the same changes in the abdominal cavity as described above. The thyroid was normal.[1]

In some cases (Nos. 1, 3, 5, 6, 8, 13) I undertook a histological examination of the inner organs after staining either with hæmotoxylin-eosin, or in the case of the adrenals, sudan. The only noteworthy observations that resulted were the following: In Cases 1 and 2 the liver was rather vascular, and, in Case 3, the liver was vascular and rich in pigment also. In Case 13 the thick-walledness and the hyaline appearance of the medium-sized vessels in the spleen were striking; besides, the pancreas was fairly

[1] On the histology of the thyroid gland in animals under thyroid feeding and under other specific diet, see Farrant, Proc. Roy Soc. Med., Path. Sect., 1914, p. 49; and Douglass, J. Path. & Bact., 1915, xix, 341; cf also MacCallum, J. Am. M. Ass., 1907, xlix, 1,158.

vascular. No kidney changes were observed. In Case 3, the adrenals, especially the right, were rich in blood and it also showed a cortex rich in pigment. Only in Cases 5 and 8 was the thyroid examined microscopically. It was colloidal and had in Case 5, a gland epithelium seemingly in two layers in places without any indication that a papillary hyperplasia was forming.

Eppinger, Falta, Rudinger and others consider the symptoms in conditions of hyperthyroidism as evidences of a state of sympatheticotonic stimulation. Biedl and others point out that some of the symptoms evidently belong to the autonomic nervous system, and, therefore, that the multitude of syndromes in hyperthyroidism can only be explained by the assumption of a polyvalent thyroidal secretion. Halsted expresses doubts as to the definiteness of our knowledge as to which symptoms are really vagotonic.

However, we cannot attempt to decide these differences this moment. Following the investigations of Langley and Cannon, I made attempts to study experimentally several questions involved. My report on the experiments, I need hardly point out, has yielded very little of a positive result. To be sure the animals on which I operated did in a few cases, as did all of those operated upon by Cannon develop, "peculiar symptoms," but it was impossible to stamp them decidedly as either sympatheticotonic or vagotonic, nor is there any agreement between the various individual cases along the lines of the operation performed. In explanation I merely call attention to the comparatively high sugar tolerance (between 15 and 12.5) in Case 7, and the very low one (1.25 to 0.63) in Case 4, not to mention such peculiarities as diffuse corneal opacity in Case 1, chronic peritoneal and intestinal changes in Cases 1 and 13, etc.

No doubt very extensive series of investigation are required in order to determine with certainty which symptoms in the cases of animals kept in long confinement may, after performing these nerve anastomoses, be referred to states of sympatheticotonic or vagotonic conditions or to hyperthyroidism.

My set of 17 cases is by no means large enough. Yet as I have not now the opportunity to complete them with new investigations, I consider that the publication of the results may be of some interest.

A few remarks may be added concerning the cause of the absence of any definite symptoms resulting from, for instance, a phrenicosympathetic anastomosis, even as I shall for the present assume, such a one being present for a long time.

Opinions have been divided as to the behavior of the different components of such hybrid nerve, as far as transmission is concerned. It may be that the nerve fibers have no inherent function in themselves but that of conduction as in the cases of the metallic wires of an electric battery. What effects are produced depends entirely on the character of the end organ to which the nerves conduct. But it is also possible that the nerve fibers themselves have some importance in determining the nature of the effect, and that they may be capable of changes with respect to this function. Langley interprets two of his experiments along the lines of the hypothesis. In one case, after uniting the severed central end of nervous lingualis (containing vaso-dilators) with the peripheral remnant of the sympathetic nerve (conducting vaso-constrictors), and on stimulation of the hybrid nerve, he obtained a contraction of the ear arteries. Similar phenomena were observed in vago-sympathetic anastomosis. Without going into the theoretical aspect of these questions, I should like to simply present a few further observations thus far made.

Rawa[1] joined the central end of the vagus with the peripheral end of the hypoglossus and observed that the former nerve thus acquired a certain degree of motor effect on the muscles of the tongue. Cushing[2] having a patient with facial paralysis, the result of a bullet-wound perforating the mastoid process, sewed up the central end of the accessory nerve after having completely severed it with the peripheral remnant of the main trunk of the facial nerve. Six weeks after

[1] Arch. f. Anat. u. Physiol., 1885, p. 296

[2] J. Nerv. & Ment. Dis., 1903, xxx, 307

the occurrence of the trauma, this operation was performed and the outcome was fairly satisfactory as far as the facial paralysis was concerned. In time the patient acquired the faculty of coördinated simultaneous motions on both sides of the median line of the head, but even nine months later, associated movements persisted in the injured half of face when the shoulder (trapezius) and head (sternomastoid) were vigorously moved.

It is of particular interest to compare with these results the observations of Langley in animals. After uniting the central end of the fifth cervical nerve with the peripheral end of the sympathetic (in two cats) he found, still after 187 days, and 112 days respectively, the ordinary symptoms of sympathetic paralysis, in the corresponding eye. But the proximal section of the hybrid nerve had not obtained any tonic effect on its distal section. And the motions of the neck and shoulder, the impulses for which normally run through the fifth cervical nerve, caused no movements in the nictitating membrane or pupil. Langley's similar experiments on a third cat, in which, after 197 days, he found that also the diaphragm had no associated movements in the pupil, have been referred to above.

It seems to me that the difference in the results found in Cushing's and Langley's cases correspond very well to the differences in the kinds of nerves that were united: in the former cases there was union of such closely related nerves as the accessory and the facial, both motor nerves; the latter's work involved joining such different nerves as those from the cerebrospinal system and those from the sympathetic nervous system. It would be an entirely reasonable conjecture that in my cases, both neck and thorax operations, such details may have played a part. But it is impossible, for the present to judge whether they have been influenced, too, in any way by the fact that, in severing the phrenic nerve, centripetal communication and transmission through that nerve were broken.

FIRST SERIES OF CASES: CERVICAL OPERATIONS

CASE A. Dog, medium sized; left phrenicus nerve sutured to left sympatheticus; under observa-

tion 111 days. Shortly before death, weighed 8,840 gms.; average pulse rate 131 (4 counts); sugar tolerance, between 10 and 5.

Other symptoms of interest[1] were loss of weight, falling of hair, some sluggishness; lessening of left pupil and lid cleft, left pupil not reacting to light.

Remarks. After operation left pupil and lid cleft were less than before, the nictitating membrane was pulled forward toward the pupil; enophthalmos. From 38 days after operation these symptoms were less pronounced. From about 100 days after the operation there was no difference in the position of the left nictitating membrane from the position of the right one. 74 days after operation the eyes were carefully examined with a flash light in a dark room; nothing especially revealed. Left pupil was always materially lessened, not reacting. Later the same results were found several times. During the last 6 weeks before killing the dog, purulent conjunctivitis was present especially in the left eye. The dog's general condition was good (until the last few days before death when he lost, during the sugar tolerance tests, 3,380 gms.). At the autopsy the suture place of the nerve was nicely healed and could not be exactly distinguished. Thyroid was rather large, transparent (colloidal). Stomach not very large.

Case B. Cat, male; left phrenicus sutured to left sympatheticus; under observation 175 days. Shortly before death, the animal weighed 4,400 gms.; average pulse rate 136 (4 counts); sugar tolerance, between 2.5 and 1.25.

Other symptoms of interest were.[1] Some hair fallen out on a spot about the size of a penny below left ear. Eye symptoms about the same as 2 months ago; the size of the pupil (left) though varying from day to day does not seem to be as small as 2 months ago.

Remarks. The usual symptoms of sympathetic paralysis were found in the left eye following the operation. From 71 days after the operation the left pupil was sometimes only a little less than the right. In spite of numerous examinations in dim light respiratory movements never were found in the left eye. Three and one-half months after operation the sugar tolerance lay between 2.5 and 1.25, the pulse rate was 135 (8 counts) the weight 4,150 gms. Left pupil and lid cleft were small; left nictitating membrane pulled forward (but no evident enophthalmos). Thus far no falling out of hair. The general condition good and remained so even at the time when the cat was killed. Autopsy: much adipose; internal organs without remarks; the nerve suture place smooth and macroscopically not to be detected.

Case C. Cat, weight 2,640 gms.; left phrenicus sutured to left superior sympathetic cervical ganglion; under observation 20 days. Shortly before death the animal weighed 1,600 gms.; average pulse rate 125 (1 count).

[1] At time of death

Other symptoms of interest. During the last week before death the general condition went down rapidly.

Thus no sugar tolerance test could be done.

Remarks. Here 2 to 3 cm. of the left sympathetic were excised below the superior ganglion whereupon the phrenicus was sewed to the ganglion itself. Left eye showed the common paralysis (yet enophthalmos was not evident and the nictitating membrane was just slightly pulled forward). During the last day preceding death there was purulent conjunctivitis in both eyes. Bladder urine at the autopsy was free from sugar. Cause of death: post-operative infection. The nerve suture had slipped. Thyroid, normal.

SECOND SERIES OF CASES: THORACIC OPERATIONS

CASE 1. Dog; right phrenicus sutured to right sympatheticus; under observation 85 days. Shortly before death the animal weighed 3,970 gms.; average pulse rate 97 (3 counts); sugar tolerance, below 10.

Other symptoms of interest.[1] Loss of weight, falling out of hair, tremor, weakness, diffuse corneal opacity during the last 2 months.

Remarks. Urine free from sugar, 2, 7, 8, 9, 60, 61, 84 days after the operation (as well as the day of the operation). The dog was quite well until 2 days before death, which was caused by pneumonia. Autopsy showed the place of the nerve suture which was nicely healed with callous-like swelling. Otherwise nothing worthy of note, except that the intestines showed occasional local scar-like extensions and the mesenteric glands were enlarged, indurated, and pigmented. Numerous firm adhesions and strands of connective tissue were present.

CASE 2. Dog; weight 6,860 gms.; right phrenicus sutured to right sympatheticus; under observation 45 days. Shortly before death the animal weighed 3,550 gms.

Other symptoms of interest.[1] During the last month before death very sensitive; during the last week very sluggish too. Loewi's test negative.

Remarks. Before operation the pupils were medium sized and equal. This fact was noted in every animal, though not always mentioned. Sugar-free urine before operation; 35 days and 45 days after operation. Cause of death, pneumonia. The point of the nerve suture healed with callous-like swelling; otherwise internal organs without findings.

CASE 3. Very large dog; left phrenicus sutured to left sympatheticus; under observation 20 days.

Remarks. Urine free from sugar at the time of the operation and 9 days after. Dog seemed to be doing quite well until he was found dead one morning. Autopsy: pneumonia. No signs of post-operative infection. The point of nerve suture nicely healed, thickened. Stomach and intestines very small. Left kidney a little larger than right.

CASE 4. Cat; left phrenicus sutured to left sympatheticus; under observation 107 days. Short-

[1] At time of death

ly before death the animal weighed 3,435 gms.; average pulse rate 133 (5 counts); sugar tolerance between 1.25 and 0.63.

Remarks. The general condition of the animal excellent all the time. No falling out of hair. Pupils equal, reacting to light. Urine free from sugar and albumin 2 months after operation and at the time of death. The cat was killed under ether. Autopsy: very much adipose (increase in weight had been stated by weighing at the beginning and at the end of the last week); internal organs without findings. The point of nerve suture without swelling, thyroid rather large.

Case 5. Young cat; left phrenicus sutured to left sympatheticus; under observation 105 days. Shortly before death the animal weighed 3,375 gms.; average pulse rate 126; sugar tolerance between 5 and 2.5.

Other symptoms of interest.[1] Loewi's test negative.

Remarks. All the time in excellent condition. No falling out of hair. Pupils equal, wide, reacting to light (3 days after operation). Urine sugar free (2 months after operation). Cat killed under ether. Autopsy: adiposity (and increase of weight) stated as in Case 4. The point of the nerve suture surrounded by adhesions between the pleural sheaths. Stomach large. Thyroid very small, non-transparent macroscopically. The follicles of the spleen very evident (young animal).

Case 6. Cat; left phrenicus sutured to left sympatheticus; under observation 100 days. Shortly before death the animal weighed 4,700 gms.; average pulse rate 113 (4 counts); sugar tolerance between 2.5 and 1.25.

Other symptoms of interest.[1] Loewi's test negative.

Remarks. Condition excellent all the time. No falling out of hair. Urine sugar-free (1½ months after operation). Some decrease in weight was noted during the last week before death. Autopsy: very much adipose. Adrenals small. Left thyroid lobe large, right lobe less and not quite as much transparent. The point of nerve suture fine, not swollen. During the narcosis (in which the animal was killed) the left phrenic nerve was stimulated with an electrical current, but without any visible effect in the opened abdomen.

Case 7. Dog, female; left phrenicus sutured to left sympatheticus; under observations 57 days. Shortly before death the animal weighed 9,500 gms.; average pulse rate 133 (5 counts); sugar tolerance between 15 and 12.5.

Other symptoms of interest.[1] Some signs of itching; falling out of hair, especially so during past two weeks.

Remarks. All the time in excellent condition. Some loss of weight was noted during the last week preceding death. Pupils equal, reacting. Urine sugar-free (2 weeks and five weeks after operation). Dog killed in narcosis. Autopsy: the point of the nerve suture not to be detected. Stomach very large, thyroid very large. Otherwise nothing to remark.

[1] At time of death.

Case 8. Cat; left phrenicus sutured to left sympatheticus; under observation 70 days. Shortly before death the animal weighed 2,850 gms.; average pulse rate 142 (7 counts); sugar tolerance between 5 and 2.5.

Other symptoms of interest.[1] Backward to the left an almost bald spot about the size of a fifty-cent piece.

Remarks.[1] The days following the operation showed a pulse rate of 135 (5 counts). Still a day after operation the urine showed sugar reaction. Two weeks before the animal was killed all the animals kept in one laboratory room (including Case 8) acquired a severe catarrhal disease of the respiratory tract and developed a bad general condition. Case 8 improved some after a little more than a week. Autopsy: adipose (especially in omentum and mesentery); the point of nerve suture nicely healed, with callous-like swelling. Internal organs without remark.

Case 9. Dog; left phrenicus sutured to left vagus; under observation 27 days.

Remarks.[1] Good condition during the past few weeks. Then loss in weight. Dog rather emaciated at the time of death. No cause of death found (dog fight?). No signs of infection. Nerve suture place nicely healed. The spleen showed an organized infarct. Stomach small.

Case 10. Dog; left phrenicus sutured to right and left vagus; under observation 87 days. Shortly before death the dog weighed 4,670 gms.; average pulse rate 108; sugar tolerance between 5 and 2.5.

Other symptoms of interest.[1] Much falling out of hair; signs of itching; thickened skin; some sluggishness during last two weeks; Loewi's test negative.

Remarks. Sugar free urine 1½ months after operation. During the narcosis (in which the animal was killed) electric stimulation of the left phrenic was made with no result in the abdomen. Internal organs without findings. Fine nerve healing.

Case 11. Cat, female; weight 2,480 gms.; left phrenicus sutured to right and left vagus; under observation 64 days. Shortly before death the animal weighed 2,150 gms.; average pulse rate 149 (3 counts); sugar tolerance between 5 and 2.5.

Other symptoms of interest.[1] Was delivered of 5 living kittens four days previous to death

Remarks. During the days following operation the pulse rate was 141 (3 counts), sugar-free urine before death as well as 3 days after. Two weeks before killing the animal the same infection developed as in Case 8. Autopsy: nothing especially to remark. Nerve suture place fine, not swollen.

Case 12. Dog; left phrenicotomy; under observations 99 days. Shortly before death weighed 11,690 gms.; average pulse rate 114 (6 counts); sugar tolerance between 5 and 25.

Other symptoms of interest.[1] Falling out of hair. Signs of itching very pronounced. Loewi's test negative.

[1] At time of death.

Remarks. General condition good all the time. Sugar-free urine before operation and 1½ months after. At the time of killing the dog, he was very thin; during the past 3 weeks preceding this, ¾ kilo loss in weight had been noted. At the operation, left phrenic and both vagi were cut; at the autopsy the vagi were quite healed together, only phrenic remained severed. Thyroid rather small. Otherwise nothing to note.

CASE 13. Dog; left phrenicotomy; under observations 73 days.

Symptoms of interest.[1] Falling out of hair; loss in weight.

Remarks. Pupils medium and equal before operation. Urine sugar free one day and also 1 month after operation and at autopsy. Good general condition. Cause of death: plueropneumonia. At the operation the phrenic, the sympathetic and both vagi had been cut; at the autopsy all these nerves were healed except the sympathetic. Changes in the intestine and peritoneum quite similar to those found in Case 1, were present. Sugar tolerance test was not done, as the dog refused to eat sugar.

CASE 14. Cat, weight 3,050 gms.; under observation 56 days.

Remarks. Sugar tolerance test, pulse count, etc., were made in this cat at the same time as in cases 8 and 11. The severe infection mentioned above which later developed interfered with the original intention to test out changes that in time might have occurred. Case 14 died before any further test had been carried out.

[1] At time of death.

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SOME REACTIONS OF BLOOD VESSELS TO CERTAIN CHEMICALS

I. ADLER

From the Department of Pathology, College of Physicians and Surgeons, Columbia University

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The various tissues and organs of the body receive from the circulating blood such material as they require for their nutrition and function; they, on the other hand, must necessarily discharge some of the products of their metabolism into the interstitial tissue and its lymph spaces. The blood vessels are therefore likely to be steeped in media more or less unstable in their chemical composition, and varying widely in different regions of the body; and it is to be assumed a priori that the vascular system reacts in certain definite ways to the physical and chemical changes that are more or less continuously taking place round about it. The study of the effects of chemical reagents, of the products of glandular secretion, of the action of drugs, upon the heart and the blood vessels, especially upon the arteries has been mainly carried out by methods of perfusion. The direct observation of the living vessels under varying mechanical, thermal, electrical, and chemical conditions, has received comparatively little attention. As early as 1852, H. Weber (43) published some experiments on the web of the frog's foot. In those ante-Cohnheim days the various theories of inflammation were much concerned with stasis in the arteries, veins, and capillaries, as a fundamental factor in inflammatory processes. Weber's experiments were undertaken from this point of view, but he mentions rather incidentally, that dilute potassium and ammonium hydrate cause contraction of the artery, but not (as he erroneously states), of the vein. He has also observed the back flow of the blood from the veins into the capillaries under

certain abnormal conditions. The brilliant researches of Cohnheim (6, 7, 8) focussed the interest of the entire scientific world for a time upon the tongue and mesentery of the living frog, but mainly for the study of inflammation and the many new and far reaching problems arising therefrom. W. H. Gaskell (10) in 1880-1882, in his articles on the Tonicity of the Heart and the Blood Vessels made use principally of perfusion, but he records also some observations upon the direct influence of certain chemicals upon the frog's mesentery. He states that solutions of sodium hydrate of 1–10,000 or even 1–20,000 contract blood vessels and that the contraction may lead to absolute closure. Lactic acid on the other hand, 1–10,000 or 1–13,000 always dilates the vessels. In proof of this he gives some measurements, based however on perfusions, and generalizes that alkalies tend to contract, acids tend to dilate the blood vessels. Recognizing the great similarity which in many respects exists between the physiological properties of the heart muscle and the smooth muscle of the arteries and veins, he develops at some length a very fascinating and plausible theory of the tonicity of the cardio-vascular system in which a dominant part is assigned to chemical control. More recently Natus (33) published a most careful study of the effects of various kinds of stimulation, principally thermal and chemical, upon the blood vessels of the mesentery and of the pancreas of living rabbits. This work was evidently designed as a basis for a theroetical discussion of stasis and its relations to the theory of inflammation, with a strong inclination in favor of nerve control as a fundamental factor in all these processes. It contains however an abundance of valuable observations to which frequent reference will have to be made. It is but fair to state that the present writer had no knowledge of the work of Gaskell and of Natus until a few months ago, when his own work was already advanced. While engaged in the study of certain problems in the pathology of arteries, it was thought useful to investigate some of the reactions of living normal vessels, especially of arteries, towards certain chemicals, not by the indirect methods of perfusion, but by direct observation under the microscope. These observations were carried on

for some length of time entirely independent of the work of others.

The great majority of these experiments were done on the frog. The frog's tongue is not a favorable object, for the massive layers of epithelium and the profuse secretion of mucus make the contact of the reagents with the blood vessels very uncertain. Alkali seemed to reach the blood vessels somewhat more readily, but acids were probably largely detained by the mucus. (Pawlow estimates that the mucus of the stomach neutralizes up to 25 per cent of its acidity, and one factor in the etiology of round ulcer of the stomach may possibly be, as Kaufmann has suggested, an insufficient secretion of mucus.) The mesentery therefore, was the object mainly employed. The frogs were either decerebrated and pithed, or curarized, and it may be stated at once that no essential difference in the reactions was recognizable, whether the central nervous system was destroyed or whether the animal was simply put under the influence of curare. The mesentery was spread over a small cork ring in the usual manner, but the spontaneous rhythmical motions transmitted to the entire mesentery from the intestines were often annoying, as they were liable to interfere with the accuracy of the measurements. The small arteries, and more still the veins, are highly sensitive to overstretching, and respond to the slightest strain beyond their normal adjustments with distortions and contractions. Complete stoppage of the circulation can be caused by incautious stretching, and when that happens it is as a rule impossible to restore the blood current even after all tension is eliminated. Resting the mesentery upon a cover glass proved no help, and was inconvenient in many other ways. All reagents were dissolved in 0.75 per cent saline. It was necessary to guard carefully against piling an excessive quantity of fluid upon the surface of the mesentery, for the mere weight of the liquid would often be sufficient to overstretch the vessels and cause serious interference with the blood current. The solutions were therefore applied by means of a small glass tube having a rubber bulb at one end and the other drawn out into a moderately fine capillary. A delicate thread of silk or yarn was drawn through the

tube, fastened at the bulbar end and allowed to protrude for the length of a millimeter or two beyond the capillary opening. With this little tube, and with the aid of an ordinary dissecting microscope, it was easy to secure direct contact with whatever part of an artery or vein one might select, and to apply the reagent in any desirable quantity. The thread, though thoroughly saturated with fluid, would nevertheless frequently cling to the vessels, and the latter, and with them often the entire mesentery, would be apt to be rather roughly dragged about. Under normal conditions and with good circulation the purely mechanical irritants that could be caused by the fine thread would have no apparent effect. It was often necessary, too, to cleanse the surface of the mesentery with some absorbent material, usually filter or litmus paper. This also might involve considerable mechanical irritation, but under ordinary circumstances would cause no disturbance. Under abnormal conditions however, even slight contact with the thread or a very gentle application of filter paper might cause vascular constriction, first always, and most readily in the smallest veins, then in the larger ones, and then in the smaller arteries, but never, in our observation, in the large main trunks at the root of the mesentery. With the magnification employed in all these experiments, nothing very definite can be said about the behavior of the capillaries. The impression was that they were not affected. Now and then a lively current might be noticed in a group of capillaries, while the arterioles in the immediate vicinity were closely contracted or showed little or no current. The reverse also was not infrequently observed, namely that the current in the capillaries ceased, while the arteries and veins were apparently quite normal. The deformation caused by purely mechanical agencies, if not too severe, and if conditions are otherwise favorable, right themselves, as a rule spontaneously and within a reasonably short time. The upper limit to which this mechanical interference can be carried without causing serious damage probably varies in different organs and also with the structure of the vessels and with the blood pressure, (Straub (38), Winkler (44). All observations were made with a Zeiss Planar lens which mag-

nified sufficiently for the demonstration of all necessary details, and having a focal distance of 20 mm., permitted all desired manipulations on the mesentery. In addition to this, a Zeiss No. 6 compensating ocular was used which contained a micrometer scale, each division of which, in this combination, was equal to about 0.025 mm. An ordinary dissecting microscope was also found useful.

ALKALIES

All alkalies tend to contract the blood vessels (let it be again understood that capillaries are not considered, except when special mention is made of them). Up to a certain limit of dilution, there is no reaction. Beyond this limit the contraction of the vessels is in proportion to the concentration. The immediate effect of the contact of the alkaline fluid with the blood vessels is usually a stoppage of the current in the arteries as well as in the veins, often with reversal of the direction of current, especially in the arteries, and oscillation of the blood column to and fro. Cohnheim has already mentioned this "*va et vient*" current. Oliver (34) and others have also taken note of this occasional cessation and reversal of the blood stream. With concentrations just strong enough to cause a mild reaction, the current recovers almost immediately and continues regularly and in its proper direction, though nearly always slower than normal, and now constrictions begin to appear. There is no evidence of any specific point of attack. The smaller veins and arteries are most easily affected, but the action of the alkali may be promptly recognized wherever the solution happens to come into contact with the vessels. By means of the capillary tube and string, it is sometimes possible to confine the action of the reagent to a single little spot on a vessel, and in that case a constriction will appear in that place only (fig. 1). This strict localization is however not often possible. The surface of the mesentery is never quite level nor quite dry. There is also more or less continuous motion, and therefore there will always be some diffusion, often in quite unexpected directions and over wide areas.

Braeuning (5), who experimented on the web of the frog's foot, has already pointed out the importance of diffusion in determining the extent to which the vascular apparatus will be affected. With weak solutions the narrowing of the vascular lumen proceeds very gradually. No definite peristaltic wave is ever to be recognized, and the direction in which the contraction advances is determined, apparently only by the laws of diffusion. If the concentration is just strong enough to produce a moderate

FIG. 1. A group of vessels at the root of mesentery. An isolated constriction of a vein touched with KOH $\frac{\text{mol}}{20}$.

effect, the contractions gradually increase up to a certain limit, but never to absolute closure, and beyond this limit the process remains stationary for some time. At this stage there appear almost regularly certain asymmetries in the outlines of the vessels. These have been noticed already by some observers, especially by Natus. The vessel is not uniformly contracted but presents a series of more or less close constrictions alternating with wider areas. In well defined cases this suggests a faint

similarity with beads on a string, and hence, for want of a better word, this peculiar phenomena is referred to in our protocols as "beading." On closer scrutiny of this so-called beading, one sees that the vascular walls are not smooth and even, but that there are areas of thickening, sometimes on one side only, more often on both sides of the wall and more or less regularly distributed along the vessels, opposite to each other. These areas of increased thickness correspond to the places of constriction, and the degree of thickening is in direct proportion to the degree of contraction. In the more advanced stages of contraction, the thickening of the walls of the vessels will, as has been stated, be uniform and perfectly even throughout the entire length of the contracted portion. It is only in the lesser degrees of shrinkage that these partial strictures appear, the mechanism of which is not quite clear. It is difficult to understand why a few circular fibers are thus seen to contract and others immediately adjoining on both sides preserve their normal tonus. It does not make any difference whether the central nervous system is destroyed or whether the animal is merely curarized.

Under the influence of the alkali the blood current as a rule is slower than normal, but it not infrequently happens, and for reasons that are not quite clear, that the current races with great velocity through the contracted vessels. With increasing concentrations the contractions become closer, so that only an extremely thin stream of plasma with cells apparently in single file is seen to pass through the arteries, while in the irregularly contracted vein the current has entirely ceased, or is extremely sluggish and oscillating jerkily backward and forward (fig. 2). It can thus come about that all the vessels, arteries, veins and capillaries of the exposed mesentery are finally collapsed and, with the exception of the main trunks at the root, completely empty or nearly so; possibly some plasma carrying an occasional blood corpuscle still trickling through here and there. If this condition is not permitted to last too long, and the concentration of the alkali has not been strong enough to directly injure the vascular apparatus, complete restitution to the normal is still possible. By removing the alkali from the surface of the

mesentery with filter paper and careful washing with normal saline, the normal circulation may gradually be restored, and it is surprising to see, when the circulation seems hopelessly gone, how it will sometimes pick up again and in course of time be as vigorous as ever. This restitution to the normal can also take

FIG. 2. Shows extreme contraction of artery and in less degree of vein, with some "beading," after application of KOH $\frac{\text{mol}}{80}$.

place spontaneously without any extraneous effort to remove the alkali, presumably when the quantity of alkaline solution and its concentration are not too great, and the normal defenses of the tissues are sufficient to cope with it. The application of stronger alkaline solutions is followed almost immediately by very close contraction of arteries and veins and complete stop-

page of the current. The vessels are empty and narrowed down to a thin thread. The vascular walls stand out quite distinctly, are apparently thicker than normal and quite even without any trace of "beading." When this stage has been reached, no recovery is possible. All efforts to neutralize the alkali by means of dilute acids or to remove it by the usual methods of absorption and washing, are of no avail, and the circulation remains irretrievably abolished. When weaker concentrations are employed, alternation of more or less close constriction and return to the normal may be repeated several times. It is to be noted, however, as Braeuning has already pointed out, that the irritability of the vascular muscle increases with each successive stimulation, and that there is thus a summation of effects; so that after a certain time, the limits of which have not yet been ascertained, the alternation of contraction and relaxation can no longer be produced and the vessels finally remain in a state of permanent contraction. Natus finds that the veins are less susceptible and respond more slowly to irritation than the arteries. In our own experiments much irregularity in this respect could be observed. Sometimes it was the vein which responded almost instantaneously to the alkali, while the artery remained unaffected for a much longer time. At other times the arteries could be seen contracted almost to complete closure, while the veins, though the current in them was sluggish almost to stasis, showed no signs of contraction. There evidently are a number of highly complex factors, leaving out of consideration altogether the functions of the nerves, which are concerned in these processes. It is probably safe to conclude that there is no difference in principle regarding the response to the action of alkalies between arteries and veins.

Nothnagel (32) in a series of well known experiments found that potassium affects the smooth muscle of the intestine of the rabbit otherwise than sodium, and he suggested that potassium probably acts directly upon the muscle, while sodium produces its effect by means of stimulation of the constrictor nerves. In our experiments no difference in the action of hydrate of potassium, sodium and ammonium upon the smooth muscle of the

blood vessels could be seen. The constricting effect, its intensity and form, appeared to depend entirely upon the hydroxyl-concentration. The lower limit of this, that is the concentration at which the very first and just recognizable, but momentary, constricting effect is visible and which often requires some additional mechanical stimulation, is in the neighborhood of pH = 9. The behavior of $NaHCO_3$ may serve as an illustration. Concentrations of $\frac{mol}{20}$ and even $\frac{mol}{10}$ distinctly alkaline to litmus cause but a very slight narrowing and beading of the veins and to a less degree of the arteries. Very frequently this constricting effect becomes apparent only after the surface of the mesentery has been slightly compressed with litmus paper for the purpose of drying and of testing the reaction. This purely mechanical irritation, which as numerous experiments have shown, does not cause any disturbance under normal conditions, will now under the influence of the bicarbonate of soda almost invariably cause a number of constrictions to appear. It is quite possible that the pressure of the litmus paper serves to bring the alkaline fluid into closer touch with the blood vessels and thus promotes the constricting effect. It should also be considered that possibly carbonic acid may be set free and that this would tend to counteract the effect of alkali. The quite unusual dark color of the blood circulating in the veins, causing them to appear almost black, whenever $NaHCO_3$ is applied, seems to speak for this, but as the hydroxyl-concentration of the solution lies between pH = 8.5 and pH = 9, this fact is in itself sufficient to account for the uncertain action of the sodium bicarbonate. A concentration of about pH = 11 seems to be necessary to induce constant, but very mild response, while forceful and close constrictions follow immediately upon concentrations of pH = 12. Our experience, as will be readily seen from this, differs from that of Hooker (19, 20), who concludes from his perfusion experiments that vascular tone is diminished by potassium and sodium ions.

ACIDS

The action of acids upon blood vessels is very much more complicated than that of alkalies. All observers seem to agree that dilute acids stimulate the blood current and dilate the blood vessels, principally the arteries, but also the veins, and probably also the capillaries (Severini (37). The peculiar arrangement of the musculature of the blood vessels admits of contraction even to complete closure, but there appears to be no special apparatus for active dilatation, so that any increase in diameter is brought about by relaxation of the contracting fibers and cannot therefore greatly exceed the normal. While the constricting effect of alkalies is fairly constant and uniform, there is not quite so much uniformity in the action of acids. Loeb (24) has long ago called attention to the fundamental differences in the biological action of various kinds of acids. On the whole it may be said of acids just as well as of alkalies, that their action upon blood vessels depends largely upon their hydrogen-ion concentration. Concentrations of about pH = 3 seem to be approximately the lower limit of dilator activity. As the concentrations progress towards the neutral limit, they become inactive, but concentrations much below pH = 3 seem invariably to cause *constriction*. It is not the purpose of the present writer to attempt an analysis of these extremely complicated chemical and physical conditions and of the disturbances of what has very recently been designated as the Vital Equilibrium (Spaeth (40). It is merely proposed to report some observations without any attempt at elaborate interpretation.

The dilating effect of very dilute acids is as a rule rather transitory, and the return to normal dimensions occurs quite promptly, as soon as the acid stimulation is interrupted; this return taking place much more quickly than the recovery after even very slight alkaline reactions. Some acids retain their dilating properties in dilutions that no longer redden litmus. It may sometimes be observed that solutions rendering the surface of the mesentery distinctly acid to litmus, will no longer show this reaction when their stimulating effect upon the circulation has

passed, and the vessels have returned to the normal. This may at times perhaps, be due to the inadvertant contamination of the surface of the mesentery with intestinal contents or with the frequently quite profuse abdominal fluid. In the case of certain acids, however, as for example acid sodium phosphate, lactic acid, and others, the tissues themselves may be supposed in all likelihood to take an active part in this neutralization. Application of stronger solutions may perhaps be followed by an instantaneous but only momentary stimulation of the current and

FIG. 3. Small artery and vein both showing considerable constriction and "beading" after application of HCl $\frac{\text{mol}}{80}$. The smaller vessel is the artery.

a dilatation almost too evanescent to be measured, but to this succeeds without exception, a more or less complete stoppage of the blood current and *constriction* of the vessels. The same uncertainty as regards the relative susceptibility of arteries and veins, that has been noticed with alkalies, obtains also with acids. Sometimes the veins are apparently more easily affected, and again the arteries seem more liable to constriction. Dilatation is certainly more pronounced in the arteries. Altogether the impression remains that the arteries respond more promptly and vigorously (fig. 3). The contractions of the vessels are

rarely as extensive as in the case of strong alkali, but are usually more or less localized, due to irregular conditions of diffusion. In further contrast to alkaline reaction, the blood vessels are not emptied and collapsed, but the current seems to stop abruptly, leaving the vessels, except in those places where there is very close constriction, filled with blood, often clotting, but not showing any signs of hemolysis. These results seem to indicate that the muscles of the blood vessels react somewhat differently to acids and alkali than the heart muscle, for Löevenhart and Eyster (26) find that the heart when perfused with alkali, contracts, but comes to rest in relaxation, while perfusion with acid dilates it, but terminates in contraction. Natus concludes that the vasodilators respond more promptly to weak stimuli, but that the vaso-constrictor effect predominates as the stimulation becomes more potent. He has not undertaken a systematic investigation of the action of acids and alkalies, but it is interesting to note that our results with acids upon the mesentery of the frog are almost identical with the effect observed by him of increasingly high temperatures upon the mesentery and pancreas of the rabbit. Dilute acids do not as a rule act as promptly as alkali. It seems occasionally as if a certain time were required for the acid to unfold its activity, though this varies very much with the nature of the acid and the degree of concentration. Butyric acid for instance, in adequate concentration, say $\frac{\text{mol}}{20}$ pH = 3, dilates and stimulates instantaneously.[1] Hydrochloric acid $\frac{\text{mol}}{1000}$ pH = 3, acts so, that an artery which may have measured 6 (these figures indicate the number of spaces of the micrometer scale), is presently, after the application of the acid, dilated up to 8, and the blood current is seen rushing through the

[1] One peculiarity of this acid ought not to pass without notice. In a certain unexplained way the frogs seemed to be extremely sensitive to this acid. It seemed to make no difference whether they were merely under the influence of curare or whether the brain and spinal cord had been completely destroyed. At each application of the acid, the animal would twitch violently, though making no attempt to wipe away the irritant with its feet.

vessels with considerably increased velocity. The application, however of $\frac{\text{mol}}{100}$ pH = 2, $\frac{\text{mol}}{20}$ pH = 1.4, $\frac{\text{mol}}{10}$ pH = 1.1 cause progressively greater constrictions and very soon complete suspension of the circulation. Sulphuric and nitric acid behave in all essentials like hydrochloric. Boric acid, on the other hand, in a dilution of $\frac{\text{mol}}{100}$ pH = 5.4 will dilate an artery 5 to 6. In much the same way have tartaric, phosphoric, acetic, oxalic, benzoic, formic acids, and acid phosphate of soda been found to behave. Lactic acid follows approximately the same rules $\frac{\text{mol}}{20}$ pH = 2.6 can still dilate an artery fron 4 to 6; concentrations below this however, act as vaso-constirctors.

A number of neutral salts proved entirely inactive. Thus magnesium sulphate and manganous sulphate in $\frac{\text{mol}}{10}$ dilution had no effect whatsoever. Hypertonic (2 per cent) NaCl solution had a very curious action. A few drops upon the mesentery caused all circulation to stop immediately. It seemed as if there remained no time for a single pulsation, and arteries, veins and capillaries, appeared filled to the utmost with blood and the picture which the mesentery presented was very much like that of an artificial injection. Calcium chloride in thin dilutions had no effect. A moderate constricting action became evident at about $\frac{\text{mol}}{20}$, but only after a period of latency varying between 4 and 10 minutes and even then the contractions developed very slowly and gradually. Barium chloride acts as a very powerful constringent in dilutions of about $\frac{\text{mol}}{30}$, the constricting action increasing in intensity with higher concentrations. $\frac{\text{Mol}}{100}$ has no effect. It takes as a rule fully 5 minutes before the constricting effect of barium becomes visible. Lead acetate and nitrate, in dilutions of $\frac{\text{mol}}{100}$ and even less, cause prompt and intense con-

traction and stoppage of the blood current. It is probable that the constricting effect of these, mostly neutral salt solutions, upon the blood vessels and the circulation, is due to the action of the kations, but the mechanism of this action is doubtless most complex and difficult to unravel. Harvey (17) warns us that "functional changes cannot be used as a criterion of permeability." It may well be that some of these substances, the strong alkalies and acids, lead, barium, and other metals, destroy the surface properties of the cells, and, as Loeb (23, 25) has suggested, enter into chemical combinations with some components of the protoplasm and thus tend to alter its properties.

Potassium iodide $\frac{\text{mol}}{20}$ though apparently neutral causes an immediate and rather violent, one might say, spasmodic, contraction which however, is only quite transitory. After a very short while the vessels resume their normal dimensions and functions. Potassium bromide acts similarly, but not quite so violently. Some hydrolysis of the salts had taken place in these solutions, but as the hydrogen-ion concentration after all, was in the neighborhood of pH = 5, it does not seem probable that the potassium causes these constrictions and one might think of the possibility of such action on the part of iodin and bromin.

For the purpose of studying these phenomena under somewhat different conditions a series of experiments was carried out according to the method initiated by O. B. Meyer (31) and developed and modified by Voegtlin and Macht (42). For further details of the method, the reader is referred to the latter publication. In our experiments a chain of 4 rings cut from the carotis of a freshly slaughtered ox, and immersed in Ringer's fluid, at a constant temperature between 37° and 38°, was attached to a lever writing upon a drum which completed one revolution in about 8 hours. The adjustment employed magnified about six and one-third times. The results obtained with alkalies in this series of experiments, coincided very satisfactorily with the observations on the mesentery of the frog. Hydroxyl concentrations of pH = 12 drove the lever upward almost instantaneously and with such force as frequently to throw it beyond the

upper margin of the drum. Lower concentrations acted proportionately less violently and more gradually. With acids our experience was somewhat different. It was impossible to obtain any definite dilation or relaxation of the tonus with any acid or with any degree of dilution. On the other hand the constricting effect of acid solutions was readily obtained and agreed perfectly with the results on the mesentery. The results obtained with various salts, neutral and otherwise, also agree well with those from the mesentery. Figures 4, 5, and 6 will serve to illustrate these findings.

It is perhaps permissible to conclude from this that alkalies and the stronger concentrations of acids, act in some way directly upon the muscle cells of the blood vessels, and cause them to contract, while the inhibitory effect on the vascular tonus, the power to dilate blood vessels to a certain extent, and accelerate the circulation, which very high dilutions of acids undoubtedly possess, seems to depend upon stimulation of the vaso-dilators. This hypothesis, if true at all, does probably not apply to the entire vascular system. The statement of Alexander Monroe (1783) that vaso-motor nerves are not to be found in the brain, has since been confirmed by some (Roy and Sherrington (36), denied by others, but the researches of Langendorff (22) speak very strongly for the chemical regulation of the blood supply in the brain and mainly by the products of its own metabolism. There is considerable evidence that smooth muscle reacts differently upon stimulation in different organs. The muscles of the stomach, intestines, ureter, uterus, etc., are probably adjusted to specific reactions upon stimulation by their own specific metabolites; by endocrine products; etc., and all of these may well act specifically also upon the special vascular apparatus of each of these organs.

As much has been written, of late, about spontaneous movements of the blood vessels entirely independent of the action of the heart (Gruetzner (12), Hasebroek (18), Homberger (13), Full (9), Severini (37), Mathes (30) and numerous others) and as the subject is still under discussion, it may be well to mention, just in passing and without any intention of entering into the

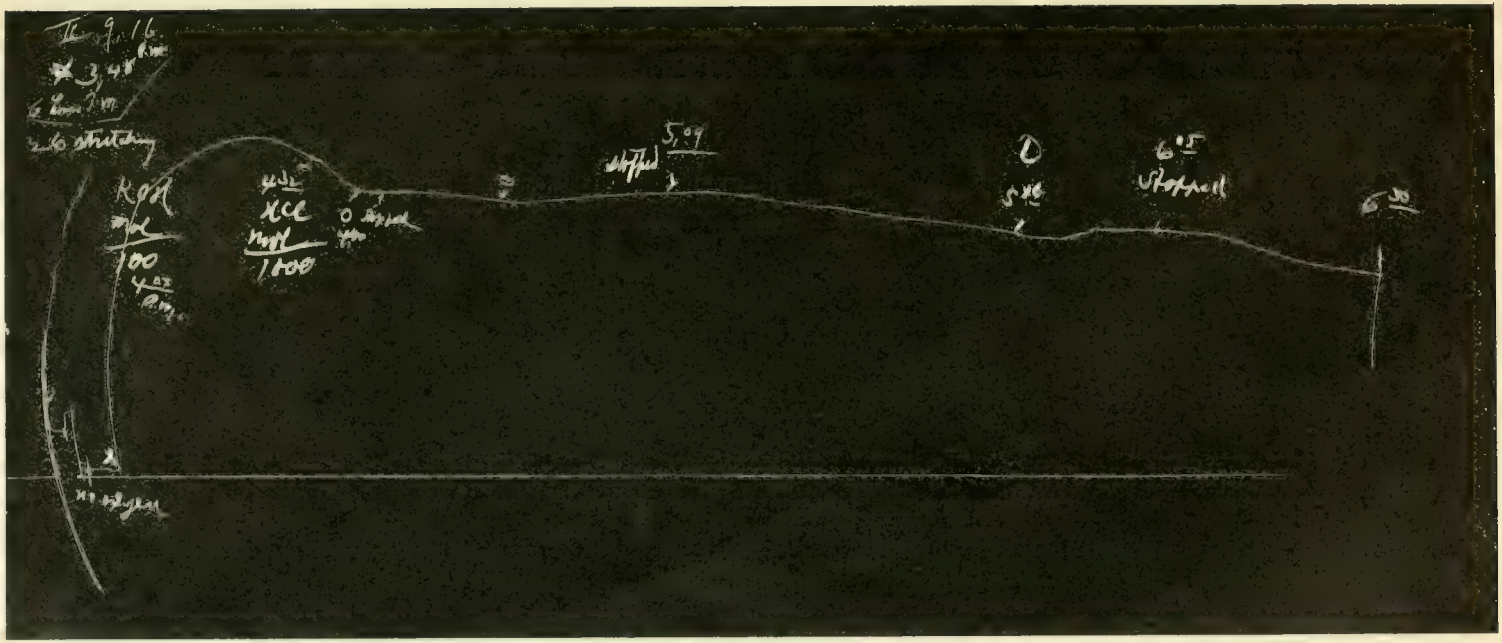

FIG. 4. Shows the prompt and vigorous contraction after KOH in concentration of pH'[2] and the absence of any relaxing effect of HCl $\frac{\text{mol}}{100}$ pH[3]

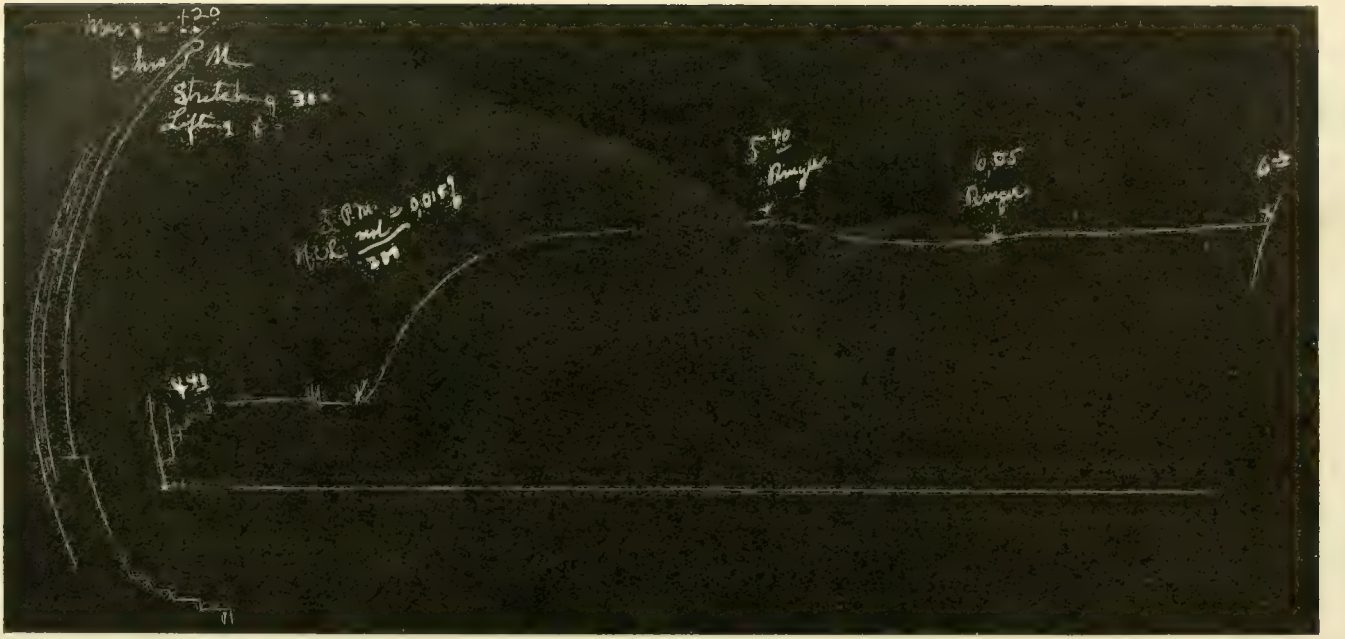

FIG. 5. Shows the gradual constricting effect of HCl $\frac{\text{mol}}{200}$.

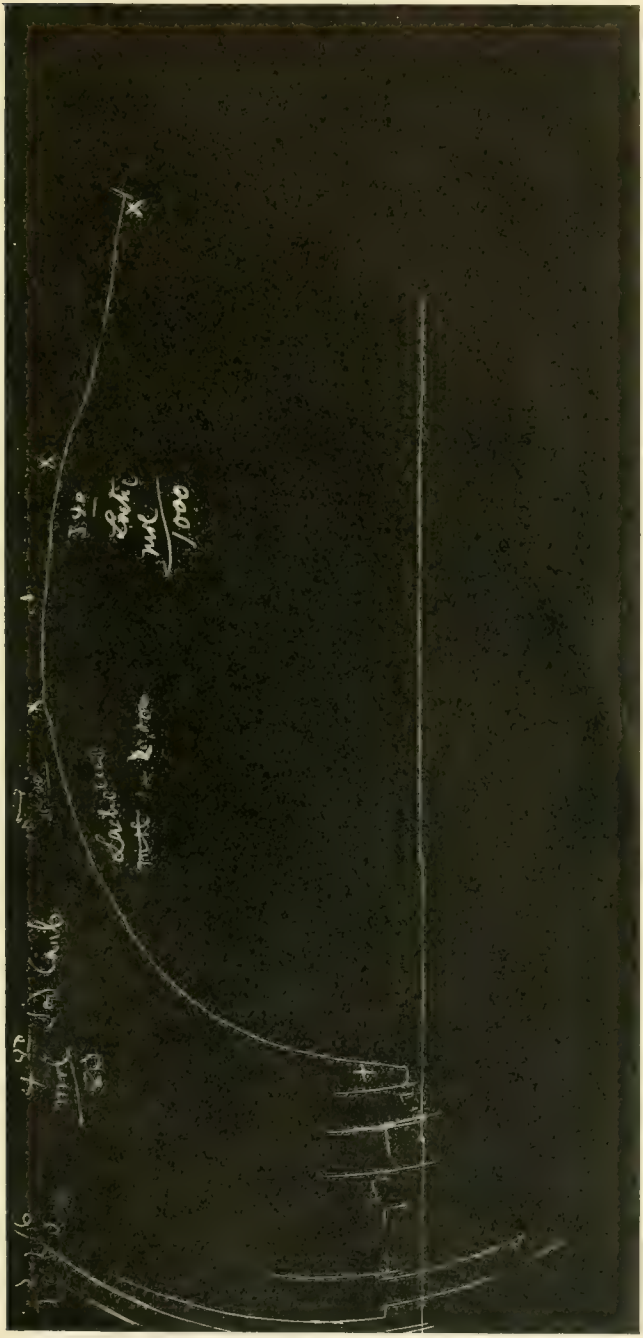

FIG. 6. Shows the prompt and vigorous contracting effect of sodium carbonate and the failure of lactic acid in thin dilutions to produce any relaxation

controversy, that it has been possible on a number of occasions to see the arteries, and in a lesser degree the veins, dilate and contract rhythmically at intervals of a fraction of a minute. The change of volume amounted at the very utmost to not more than 1 space of the micrometer scale. The conditions which might favor these spontaneous movements were not investigated.

As a further illustration of the methods employed some few and characteristic of the very numerous protocols are subjoined.

Experiment 56. Frog decerebrated and pithed. Artery measures about 5. Application of sodium hydrate $\frac{\text{mol}}{20}$. After about 7 minutes artery measures 3, while the veins which have been much narrowed and have shown considerable "beading" are recovering. After another 3 minutes the artery beginning to dilate, another application of the same solution in the same place. Again after 3 minutes the artery is considerably beaded, measuring at the narrowest places less than 1 and in the interspaces about 3. The adjacent veins also much narrowed. The current in the artery almost completely stopped. Washing with normal saline. After about 6 minutes, veins almost normal, arteries still beaded and about same measurements. Lactic acid $\frac{\text{mol}}{200}$ pH = 3.08 applied. After 2 minutes the current in the arteries is distinctly more lively and the constrictions very slowly begin to loosen. Within the next 4 minutes, 2 applications of the same solution of lactic acid are made. The point of greatest constriction has dilated from 1 to about·3 and the interspaces to about 4. Some of the capillaries are resuming their current. After 10 minutes artery has slowly almost regained its normal measure and the capillaries are active. The field is carefully dried with litmus paper and a local application of potassium hydrate $\frac{\text{mol}}{20}$ is made to another artery which shows immediate slowing of the current and narrows down to almost filiform for some distance above and below the point of contact. Circulation in capillaries has stopped. Current in the constricted artery is swift, but the lumen of the vessel which originally was a little more than 5 has narrowed down to about 1 without any "beading." After 7 minutes dilatation is beginning, but considerable "beading" appears. After another 3 minutes the artery is recovering, lumen almost 3.

Washing with lactic acid $\frac{\text{mol}}{200}$ = pH 3.08. Artery dilates almost at once, the current in both arteries and veins is very lively, in the capillaries it is slowly being resumed. After 10 minutes, another washing with lactic acid and after 3 minutes more, the potassium artery is fairly normal, the sodium artery still shows some signs of constriction.

Experiment 68. Frog is curarized. Artery selected measures between 5 and 6 just above bifurcation. A vein to the left measures 5, to the right one that measures 4. Application of $\frac{\text{mol}}{20}$ neutral calcium chloride, the current is somewhat slower. After 5 minutes another application of the same solution without any effect. After 6 minutes the mesentery is dried with litmus paper which has no effect upon the current or the measurements of the arteries and shows no reaction. After 4 minutes application of $\frac{\text{mol}}{20}$ neutral magnesium sulphate. After another 5 minutes another application of the same solution. After about 17 minutes there is no change. Measurements are the same, blood current even in the capillaries is excellent. The mesentery is dried with litmus paper which shows neutral reaction, and there is no effect upon the current or the vessels. After about further 8 minutes, conditions being perfectly normal, application is made of potassium iodide $\frac{\text{mol}}{20}$. Within a minute the blood current racing along with considerable velocity, the artery is violently constricted from the periphery towards the center. At the original point of measure. it is barely 3. The vein is hardly affected. After about a minute, the artery has practically assumed its normal dimensions. After 5 minutes another application of the same solution of potassium iodide was made and immediately again the artery is narrowed from periphery towards the center so that at point of greatest constriction, it measures a little more than 1. The vein again does not seem to be touched. Within a minute the artery begins to dilate again, but the recovery this second time is not as rapid as at first. 10 minutes later everything is normal, the artery having long before regained its normal dimensions. The surface of the mesentery is dried with filtering paper without any effect upon the current or the vessels. $\frac{\text{Mol}}{20}$ potassium bromide is applied. Immediately thereupon there is a very slight narrowing of the artery and also of the vein. The blood current everywhere is perfect. After

another 5 minutes the vein having now recovered, another application of the same solution of potassium bromide is made. Again there is an immediate narrowing of the vein and also a very slight, hardly measureable, narrowing of the artery. After 10 minutes the artery has recovered completely, but the vein is still considerably narrowed measuring over a great part of its length barely 2, and its current is very sluggish. The mesentery is dried with filtering paper which has no disturbing influence. After 5 minutes the vein is no longer uniformly narrowed. but begins to dilate, showing still considerable constriction. The artery and capillaries are normal, and the blood current everywhere good. Application is made of formic acid $\frac{\text{mol}}{100}$ pH = 2.9. After 2 minutes artery is distinctly narrowed, now barely 3, with considerable "beading" throughout its entire extent. The vein is slowly dilating. After another 3 minutes, the vein is still not normal, though dilating, the artery about the same, the current very swift. Another application of the same solution of formic acid. There is no further narrowing of the artery which is much beaded but seems as if it were beginning to recover. The vein is still slowly dilating. All the capillaries are working. After another 5 minutes the vein is almost normal, the artery undoubtedly recovering, having in some places its normal measurement and at places of greatest constriction, now measuring about 4. Blood current in arteries, veins and capillaries, excellent. Experiment which has lasted nearly 2 hours closed.

Experiment 80. Frog decerebrated and pithed. Normal measure of artery a little more than 6. Hydrochloric acid $\frac{\text{mol}}{100}$ pH = 2 applied and within 1 minute, artery is nearly 8. After 4 minutes again normal dimensions. After wiping with blue litmus which fails to show acid reaction and application of normal saline, artery is nearly 7. A second application of $\frac{\text{mol}}{100}$ hydrochloric acid and within a minute artery nearly 8. Current in the small capillaries seems occasionally to stop until a small lump of agglutinated red cells is thrust through with great great force when the current becomes normal again. This process is repeated again and again but gives place to normal conditions after about 5 minutes. The artery now has its normal dimensions. Blue litmus is not reddened. Normal saline is applied and the artery narrows somewhat. After several minutes, application of $\frac{\text{mol}}{100}$ sodium

hydrate is followed immediately by slight beading of both artery and vein. After 3 minutes artery 5. After another 6 minutes application of $\frac{\text{mol}}{100}$ hydrochloric acid. Current at once becomes more rapid and artery normal. Beading has disappeared, but after another 2 minutes the artery narrows down to 5. After still another 3 minutes the surface of the mesentery still reddens blue litmus and the artery remains at 5. 6 minutes thereafter $\frac{\text{mol}}{80}$ sodium hydrate. Artery is reduced to 4 with pronounced beading. After a couple of minutes the artery spontaneously dilates to nearly 5. In the course of the last 10 minutes, it can be observed that without any external interference there is a rhythmical contraction and dilatation of the artery alternating at half minute intervals. After another few minutes application of $\frac{\text{mol}}{25}$ hydrochloric acid. Current in capillaries stops immediately, but beading disappears, the vessels begin to dilate and after about 2 minutes the artery measures 6, but after another 2 minutes an entirely spontaneously narrowing begins so that after about 7 minutes, the artery measures not quite 4. 15 minutes later washing with normal saline which causes immediate but temporary stoppage of all current and a narrowing of the artery to a little over 3. About 10 minutes thereafter the current in arteries and veins has regained normal velocity but in the capillaries, it has not yet recovered. The measurement of the artery is now a little more than 5 and remains so. Experiment has lasted about 2 hours.

Experiment 71. Frog decerebrated and pithed. Brilliant current in arteries, veins and capillaries. In order to test the effect of mere mechanical irritation, a thread that had never been used, but was simply moistened with normal saline is employed to stroke and push about some of the larger arteries and adjoining veins for a greater part of a minute. No effect was visible on the artery, but some of the veins responded by some narrowing and constriction, but no beading. This purely mechanical effect passes very promptly and in a very short time the vein resumes its normal dimensions without any alteration in the current. As soon as everything had become entirely normal, a local application is made to one of the larger arteries of $\frac{\text{mol}}{20}$ barium nitrate. The current at once both in the artery and adjoining veins becomes slow and oscillates. There is no current in the surrounding capillaries.

In about 2 minutes a number of veins collapse and become almost entirely empty. It takes fully 5 minutes however before the artery shows any effect, then there is almost complete closure and the current is extremely slow. After about 5 minutes washing with normal saline, but the constriction goes on and the artery now, about 10 minutes after the application of the barium, is simply a straight line with a thin thread of red corpuscles in single file passing through it. There are constrictions of various degrees in the veins. 15 minutes thereafter the effect of the barium is still unaltered, the current extremely sluggish and more or less contraction of all vessels. The surface of the mesentery is dried with filtering paper and again normal saline applied. After another 15 minutes the original artery is almost normal, the veins less so, but much improved. The current is very slow and more or less irregular. Application is made of $\frac{\text{mol}}{1000}$ lactic acid pH = 3.4. In about minute the current is decidedly stimulated, the constricted veins are beginning to dilate and some of the capillaries which have been empty are showing current again. After another 5 minutes another application of $\frac{\text{mol}}{1000}$ lactic acid is made. The current remains good and the surface is dried after a while with filter paper and washed with normal saline. After about 15 minutes, the current everywhere is somewhat slow, but fairly regular. The mesentery is again dried with filter paper and a local application is made of a 2 per cent solution of NaCL. Almost instantaneously the current in the capillaries, arteries and veins, large and small, stops completely without any constriction, so that all the vessels are gorged with blood and the mesentery looks as if it had been artificially injected. After 5 minutes, the mesentery is dried with filter paper and then washed with normal saline. After about 10 minutes the circulation appears to be starting up again in some of the arteries and veins and in many of the capillaries, and it seems that if time were given, the circulation would recover.

Summing up results the writer is fully conscious that there still remain sufficient problems that have not been solved and discrepancies that have not been reconciled. The curiously uneven and apparently irregular contraction of the vascular walls which we have called "beading," demands further investigation and explanation. The fact that cleansing the mesentery with filter or litmus paper in one instance causes no disturbance what-

soever, and that again violent constrictions may follow the same manipulation, though suggesting several plausible hypotheses, has not attained a precise explanation. Similarly it has often been observed that acid solutions (alkaline not so frequently) had apparently no effect until the mesentery was washed with normal saline, when the constrictions promptly appeared. Here again several explanations suggest themselves, but all these problems await further investigations. Nevertheless it is believed that certain conclusions may safely be drawn from our observations. They furnish a further corroboration, of what may now be accepted as a fact, that "there is, in the words of Bayliss, a complete chemical regulation of the cardio-vascular system which may act independently from the central nervous system." It has here been shown also that it is not only the blood circulating in the blood vessels, that takes part in the regulation of their functions, but that the chemical conditions of the tissues and fluids surrounding the blood vessels also exercise an important influence. It appears furthermore that it is not only the products of the internal secretions which are concerned in this, but that common acids and alkalies; the specific metabolites of the tissues and cells; besides a number of neutral salts, under ordinary circumstances foreign to the organism, may exercise a controlling influence. Strong acids act like alkali in compelling vessels to contract. It is true, that such extreme chemical changes as have here been presented are ordinarily not likely to occur. Rowntree (27) and his associates estimate the hydrogen-ion concentration of normal human blood plasma as varying from pH = 7.4–7.6; of the blood serum from pH = 7.6–7.8. In clinical acidosis pH = 7.3–7.1 and in dogs just before death from experimental acidosis pH = 6.9. Henderson (21) has shown that the blood is able to dispose of considerable quantities of alkali or acid without much change in its reaction. There must necessarily however be a limit to this, and one may conclude from the observations here recorded that besides the chemical and physical reactions of the blood itself, there are other chemical and physical factors which, when once a certain limit has been overstepped, may lead to grave disturbances of circu-

lation, and one can easily conceive of certain pathological processes such as inflammations, thromboses, necroses and other local, possibly even systemic, affections originating in this manner.

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TISSUE CULTURES IN THE INVESTIGATION OF CANCER

ROBERT A. LAMBERT

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City

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It is recognized that progress in many departments of science is dependent in large measure, if not entirely, on the development of improved methods of investigation. In the field of cancer research it has become increasingly evident in recent years that new methods of attack must be worked out before further important advances can be expected.

The discovery of the factors concerned in the unlimited lawless growth of cells constitutes, obviously, the main cancer problem. It would appear therefore that the recently devised method of cultivating tissues *in vitro* (Harrison, Burrows) by which we are enabled to study isolated somatic cells under conditions which allow the introduction of known and measurable factors into experiments, should mark an important advance in the attack upon this fundamental question. Furthermore, since it is possible to subject cancer cells, thus isolated, to various chemical and physical agencies, it may be suggested that we have also in the new method a possible means for solving another great cancer problem, namely, the treatment of malignant growths.

In the study of human tumors, the method will probably fill a great need, since it is not possible to secure the continued propagation of such tumors by transplantation, by the methods pursued in the case of tumors of certain of the lower animals.

The purpose of this paper is simply to review briefly a few of the results which have been obtained with tissue cultures in studies upon cancer and related subjects, and to suggest some problems in which it seems probable that the method may be

advantageously applied. It is possible that a review of the results thus far obtained may prove disappointing. But in palliation it should be said that the method is young and in many respects still imperfect; and that much time and energy have been expended by a number of workers in the past five years toward improving the technique so as to permit the cultivation of a greater variety of tissues over longer periods of time.

Details of the history of the method of tissue cultivation *in vitro* and its development will be found in various papers to which references are given at the end of this article (Loeb (1), Harrison (2, 3), Burrows (4, 5). For the convenience of those interested, a brief review of the technique of the method is given here.

TECHNIQUE

The simplest and most satisfactory form of culture consists of a small piece of tissue placed in a thin drop of plasma on a cover glass which is inverted and sealed over the cavity of a hollow-ground slide. To obtain the plasma in such a way that it will remain unclotted until the tissue is added, several precautions must be observed. The blood must flow directly into the container (preferably a small test tube) without contamination with tissue juices, and should be kept cold in oiled or paraffined tubes both during and after centrifugation.

Blood is obtained from human beings or large laboratory animals (dogs, goats), by puncturing a superficial vein with a needle of large bore, previously boiled in albolene or olive oil, and allowing the blood to flow directly from the needle into paraffined tubes set in large centrifuge cups which are filled with cracked ice.

Small animals such as mice, rats, and frogs may be bled from the heart in the same way. The writer has devised another method (7), however, for bleeding small animals, which does not entail the sacrifice of the animal as does bleeding from the heart, a distinct advantage in certain experiments where it is important to obtain plasma from the same animal more than once. The carotid, jugular, or some other accessible vessel is exposed and

clamped lightly at two points some distance apart; the vessel wall, after clean dissection, is caught by delicate forceps at a point midway between the clamps in such a way that the lumen is not occluded; the vessel is then severed beyond the forceps, the proximal clamp released and, with the end of the vessel held

FIG. 1. Method of bleeding small animals for obtaining plasma. The artery is held away from the tissues and the blood allowed to spurt into paraffined tubes

away from the tissues, the blood is allowed to spurt free into cold paraffined tubes as before (fig. 1). Even so small an animal as the mouse may be bled several times in this way.

On account of the dryness of their tissues and the proximity of the wing vessels to the skin, fowls and pigeons may be bled di-

rectly from the wing vein by making a clean incision through the skin and vessel, and allowing the blood to drip from the surface into the tubes.

Blood obtained by any of the methods mentioned is centrifuged at high speed for several minutes, and the supernatant plasma is then put aside in a cold place, or packed in ice.

The length of time that the plasma may be preserved uncoagulated varies greatly with different species. Fowl and pigeon plasma may be kept indefinitely if properly handled. On the other hand, rat and guinea-pig plasma will rarely remain fluid longer than two hours, often not so long. Human, rabbit, dog, and cat plasma may be kept for several hours at least, and sometimes even for days.

The remainder of the technique consists in placing small pieces of tissue, 0.5 to 2.0 mm. in diameter, on a cover glass, and adding immediately a small drop of plasma. The cover glass is inverted without delay over a hollow-ground slide, sealed with vaseline, and incubated at body temperature.

It should be stated in this connection that tissue cells show no such proliferative capacity in cultures as do bacteria. Furthermore, it has not been possible as yet to obtain an active growth of any of the highly differentiated body cells, though certain specialized elements, such as ganglion cells, may survive for a time.

The cultivation of human tissues has offered special difficulties, owing to the fact that the fibrin in the clotted human plasma, which forms the necessary scaffolding for the outgrowth of cells, is regularly digested by the tissue fragments, and the cells, not being able to wander out into the medium, soon die. The writer has overcome this difficulty by using as a culture medium a mixture of human serum (or plasma) and chick plasma, the fibrin of which resists digestion. (It was found that chick plasma alone did not form a satisfactory medium.)

That the method is in many respects surprisingly simple should be emphasized. It has been little used in cancer laboratories, a fact which is probably to be attributed to supposed difficulties in technique (danger of contamination, maintenance of

a uniform temperature during preparation of cultures, etc.), points which have certainly received unmerited emphasis in several papers (6). As a matter of fact, practically no effort at all is required to avoid bacterial contamination; thus, the writer has used tissue from the liver of a human cadaver four hours after death (metastatic tumor nodule lying directly on the colon), and observed not a single colony of bacteria in a large number of preparations. It is possible to use even such a tissue as skin, where a certain number of bacteria are known to be present, if partial sterilization with weak alcohol be first carried out. The plasma itself, especially that of fowls, evidently possesses a definite bactericidal property, by which a certain number of organisms are taken care of. It is not necessary to keep the tissues at or near body temperature while preparing the cultures, and within certain limits the time elapsing between removal of the tissue from the living body and the preparation of the cultures is not important. The tissue of the adult rat may be used after several days' preservation at room temperature or in the ice-box, and the writer has obtained successful growths of human tissue kept for six days in cold storage. Embryonic tissues are capable of longer preservation—five to eighteen days, the length of time depending on the temperature.

The problems in which the method of tissue culture has been employed by the writer may be conveniently discussed under several headings: (1) Comparative study of the behavior of cancer cells and normal cells growing *in vitro;* (2) Cancer immunity; (3) Stimulation of cells growing in cultures.

COMPARISON OF THE BEHAVIOR IN VITRO OF NORMAL CELLS AND CANCER CELLS

The similarity between the growth *in vitro* of sarcoma and connective tissue on the one hand, and between carcinoma and normal epithelium on the other, has been emphasized in previous papers (7, 15). Sarcoma and connective tissue cells wander out singly or in chains, while epithelial cells, normal or neoplastic, tend to spread out in sheets or groups.

Certain differences which may not be without importance have been observed in the behavior of normal and malignant tissues. In the first place, a greater motility characterizes the cancer

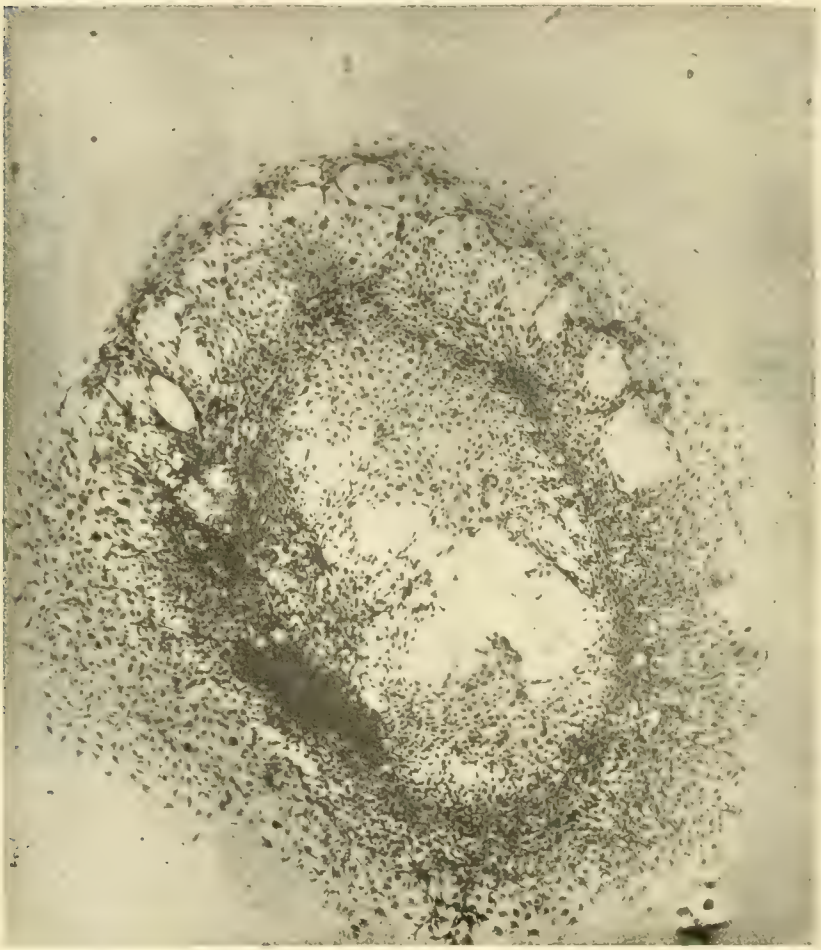

FIG. 2. Mouse sarcoma, ten-hour culture, showing extensive outwandering of cells which began within two hours after preparation was placed in incubator.

cell, especially the sarcoma cell, as compared with the corresponding normal element; a sarcoma cell may often be seen traveling through the medium at a rate almost equal to that of polymor-

phonuclear leukocytes (figs. 2 and 3). This fact, as has been pointed out before (22), probably throws light on the mechanism of the invasive growth and spread of cancer in the body. It is not necessary to regard the formation of metastatic tumor nodules as always a result of the passive transportation of cells from a primary tumor by the blood or lymph stream, when the cells may easily get from place to place by their own powers of locomotion. The writer has calculated that a tumor cell, if it survived the trip, might make its way from the middle of the breast to the axilla in less than four weeks.

A second important, and somewhat surprising difference between normal and tumor tissues lies in the fact that the continued propagation of certain normal cells, especially those of connective tissue, is, as a rule, much easier than in the case of tumor cells. Many carcinomata and sarcomata, especially those of human beings, will not grow even in primary cultures, while others, rat and mouse tumors, for example, grow actively for a few days but tend to die out even when transferred early to fresh plasma. On the other hand, it has been the writer's experience that connective tissue becomes very much more active in subcultures, so that after several transfers a most active cell multiplication is observed, dozens of mitotic figures being sometimes seen in a single preparation (fig. 4). The explanation for this difference in behavior is not obvious. It may be suggested either that some particular substance necessary for tumor growth is not being supplied in sufficient quantity in cultures or, stated in another way, that the cancer cell is really, in a sense, a highly differentiated cell which is particularly susceptible to changes in food supply and environment. That the cancer cell is a cell of lowered vitality is suggested by the results of some studies made by the writer on the comparative resistance of tumor cells and normal cells to heat (8). When normal connective tissue elements and cells from mouse or rat sarcomata were subjected to different degrees of temperature above that of the body, for variable periods, it was found that the latter were definitely more susceptible to heat than were normal actively growing connective tissue cells. Similar experiments with human tissues have

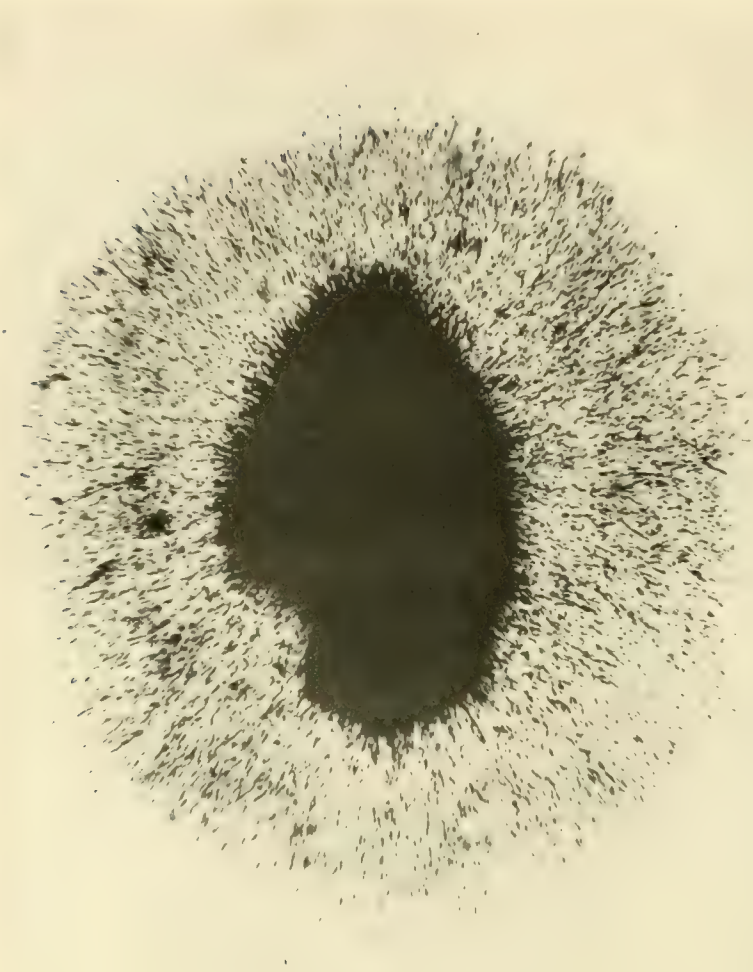

FIG. 3. Rat sarcoma, five-day culture, showing cells with numerous pseudopods creeping along the cover glass.

FIG. 4. Rat connective tissue cells, twenty-four days *in vitro*, showing very active multiplication after third transfer. Thirty mitotic figures were counted in the preparation. The tissue used was a piece of blood-vessel from an adult rat.

recently been attempted, in the course of which the writer has found that human connective tissue cells and wandering cells are quite as resistant to high temperatures as are those of the rat; a satisfactory comparison with human tumor tissue has not been made, however, on account of the difficulty experienced in obtaining sarcoma suitable for cultivation.

That the cancer cell is a particularly hardy cell, and that malignancy is due in some measure at least to this property, is a popular notion. This idea has, indeed, received some support from studies on the resistance of certain rat and mouse tumors to freezing and to toxic chemical substances (9, 10, 11). It is to be noted, however, that these studies, with one exception, were not comparative. The writer has found that many types of normal tissues isolated in tissue cultures are, like tumor cells, very resistant to low temperatures (12) and to many chemical agents. Ribbert (13) has called attention to the tendency of tumors to necrosis, maintaining that cancer cells are very susceptible to injuries of many kinds, and that this state of low vitality is referable to their poor vacularization.

While not accepting Ribbert's explanation, the writer is inclined, as the result of his own studies, to endorse the view that the cancer cell is a cell of relatively low resistance and that malignancy is to be attributed entirely to the capacity of the cells for unlimited growth.

CANCER IMMUNITY

The results of the use of tissue cultures in the study of cancer immunity have been described fully in previous papers (14, 15, 16, 17) and need only be summarized here.

Although recent studies have shed new light on the question, the problem as to the exact nature of the resistance to transplantable cancer is still unsettled. Lambert and Hanes found that rat sarcoma cells will grow quite as well in the plasma of an immune rat (naturally or artificially immune) as in the plasma of a normal rat, an observation which affords further evidence that cancer immunity is not to be attributed to circulating antibodies of a cytotoxic nature.

It was shown further that the plasma from an animal of a foreign species (guinea-pig or rabbit) may serve also as a good medium for the cells. The fact that rat sarcoma cells can grow in such an alien plasma for at least thirty days would appear to invalidate Ehrlich's hypothesis of athreptic immunity.

On the other hand, it was discovered that if the guinea-pig or rabbit be first immunized against rat tissues by suitable injections of rat blood, skin, or tumor, the plasma of the treated animal becomes no longer suitable for the growth of rat cells, but is on the contrary distinctly toxic for any rat tissue. This experiment, besides showing that antibodies for tissue cells can be readily demonstrated in tissue cultures, proves also that cytotoxins are not specific for the cells used for immunization.

In further efforts toward demonstrating a cancer immunity reaction *in vitro* Dr. Edna Steinhardt and the writer attempted to cultivate rat sarcoma cells in immune rat plasma in association with normal tissues from immune animals (spleen, lymph-node, liver, and leukocyte emulsion), with the idea that the immunity phenomenon might be the result of the combined action of immune serum and some tissue or organ. We found that the growth of the tumor cells *in vitro* was not influenced by such a combination of factors. This negative result may have been due, however, to faulty technique, on account of which the essential protecting cells may have been injured, or present in insufficient number. In view of the recent work of Murphy (18) who attributes immunity against cancer to the action of certain lymphoid elements, it should be possible, by a proper combination of tumor cells and lymphocytes, to obtain an immunity reaction *in vitro*. Indeed it may even be suggested that the method of tissue cultures should serve to settle some of the questions which have been raised by this new work.

STIMULATION OF GROWTH IN VITRO

This discussion of the application of tissue cultures to cancer problems would not be complete without reference to efforts directed toward the stimulation of normal and tumor cells, when

freed from body restraint. Without entering into a detailed discussion of physical and mechanical stimuli, the writer wishes again to emphasize the fact (19) that variations in the depth of the hanging drop and in the density of the fibrin meshwork (which can be altered by dilution) influence markedly the extent to which actively motile cells wander outward.

A single recent observation on a culture of human connective tissue exposed to a temperature of 45° for a few minutes may be of interest in this connection. Examination of the specimen just before exposure showed only two dividing cells in the entire preparation; ten minutes after heating, however, at least a dozen cells were seen in early stages of mitotic division. It may be that this single observation is without significance, but it does suggest further work on the effect of sudden elevation of temperature upon cell multiplication.

The action of various tissue extracts on growing cells has been studied by Dr. Carrel and his co-workers (20, 21), and very striking results have been reported on the effect of such preparations, especially those of chicken sarcoma, on embryonic chick tissues. The writer has not been able to verify their findings in such of the experiments as have been repeated, and is inclined to the opinion that the results of these authors are to be explained in other ways. It is certain that they do not possess any general application. An extract of human tumor, recently employed by the writer, appeared to inhibit rather than stimulate the growth of normal human cells.

Results following the use of certain chemicals (Scharlach R. and Sudan III) which have been shown to cause an abnormal proliferation of various tissues when injected into animals, have been disappointing. Cultures of chick embryo, and of rabbit skin and cornea, were treated with these dyes through several subcultures with no apparent effect, although they were applied in different ways and in varying strengths.

In spite of these negative results, this particular field seems to offer great possibilities. Since it is known that some sort of stimulus, or possibly the loss of some restraining influence does start cells within the body on a career of reckless multiplication,

it seems possible that patient work in this direction upon cells growing outside the organism may supply the key to this phase of the cancer problem.

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A COMPARISON OF THE RESULTS OBTAINED BY THE COLORIMETRIC AND GRAVIMETRIC DETERMINATIONS OF CHOLESTEROL.

By J. HOWARD MUELLER.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

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It has been observed in the course of a considerable number of cholesterol determinations made by the colorimetric method of Autenrieth and Funk,[1] and the digitonin method of Windaus[2] (gravimetric) that the averages of values obtained were considerably higher in the colorimetric than in the gravimetric analyses. When this fact became appreciated, check determinations were undertaken on the same specimens of blood, and it soon was evident that there was a real source of error in one of the methods, since there was invariably a difference of as much as 10 per cent and often more, the colorimetric results being higher. In a new modification of the colorimetric determination recently published, Bloor[3] has claimed still higher results than those obtained by the Autenrieth-Funk method, by an average of 20 per cent. Grave doubts were thus cast on the reliability of the digitonin method. Since, however, this is the only available means of determining separately the free and combined forms of cholesterol it seemed highly desirable to undertake a critical study of the two procedures and to attempt to locate, and, if possible, to rectify the error. To this end it was necessary to study both the actual technique of the determination and the method of extraction of the cholesterol from the tissues.

In the literature there is comparatively little which might throw any light upon the question. Autenrieth and Funk[1] quote one analysis in which

[1] Autenrieth, W., and Funk, A., *Münch. med. Woch.*, 1913, lx, 1243.

[2] Windaus, A., *Z. physiol. Chem.*, 1910, lxv, 110.

[3] Bloor, W. R., *J. Biol. Chem.*, 1916, xxiv, 227.

practically identical figures for total cholesterol in the blood were obtained by their colorimetric method and by the digitonin method. For the digitonin method, however, the blood was dried with plaster of Paris, and after grinding, extracted for 6 days in a Soxhlet with ether. Since it is improbable that ether ever brings about a complete extraction of cholesterol from dried blood (see below), it is a little hard to understand how this could have been so. Csonka[4] gives an isolated instance where gravimetric and colorimetric determinations agreed quite well, the blood having been first digested with alcoholic potash before extraction. Weston[5] investigated the gravimetric method and obtained results which were much *too high* (by about 9 per cent) when a weighed amount of cholesterol was added to blood, and the latter then extracted by the method devised by him.

The colorimetric methods, of which there are several modifications, are fairly simple, and if accurate, should be of considerable value where nothing more than a determination of total cholesterol is required. It is true that quantitative methods involving colorimetry are criticized by many as introducing a considerable degree of personal error. The green color, however, which is produced in the Liebermann-Burchard test for cholesterol is a most satisfactory one to measure with a colorimeter, particularly if from 1 to 4 mg. of cholesterol are used. Even in those modifications where only 0.3 to 0.5 mg. are used, the color can be compared with a fair degree of accuracy, though not so easily as with the larger amounts. It thus seems desirable to carry out the colorimetric test with the entire extract of the 2 or 3 cc. of blood used, rather than to dilute it to 100 cc. and use a 5 or 10 cc. aliquot.

In order to check the personal error in using a colorimeter with this color, the following experiment was done.

From a stock solution of Merck's cholesterol in chloroform, containing 0.0030 gm. in 5 cc., four unknown solutions were prepared by an associate, using varying quantities of the standard plus enough pure chloroform to bring the volume up to 5 cc. 2 cc. of acetic anhydride and 0.1 cc. of concentrated sulfuric acid were then added to each tube, and also to a standard tube containing 2.5 cc. of the stock solution and 2.5 cc. of chloroform, and thus 0.0015 gm. of cholesterol. After the color had developed, a

[4] Csonka, F. A., *J. Biol. Chem.*, 1916, xxiv, 431.
[5] Weston, P. G., *J. Med. Research*, 1913–14, xxix, 457.

series of ten readings was made for each unknown solution, using a Dubosecq colorimeter, with the standard set at 10 mm., the average taken, and calculations were made. The following results were obtained:

Cholesterol.

Taken. gm.	Found. gm.
0.00060	0.00056
0.00180	0.00178
0.00240	0.00241
0.00108	0.00086

The agreement in the first three cases was excellent. The fourth tube, on the other hand, shows a rather great error, but it may serve to illustrate a difficulty which is occasionally, though seldom, met with; namely, the development of a green color of a markedly different shade from that produced in most cases. Such a variation appeared in the fourth tube of the above experiment, and undoubtedly accounted for the error. Why this should have occurred in a tube containing only pure cholesterol and chloroform is not clear—possibly from the presence of a little dirt. Autenrieth and Funk[1] state, however, that it is not infrequent to have a green color develop containing a shade of yellow or brown, but that this does not interfere with the values obtained by the colorimeter. That a difference in the character of the color in an unknown and in the standard should cause an error is only natural, and this is illustrated above. Determinations made on blood in this laboratory have only infrequently shown this variation in color. It is possible that when present, it results from the use, without proper decolorization, of a chloroform extract which itself has a more or less marked reddish or yellowish brown color. This is practically always true of the chloroform when it first separates from the alkaline blood mixture. Autenrieth and Funk[1] say this can be removed simply by shaking the chloroform with dry sodium sulfate, which dehydrates and at the same time removes the color from the extract. This has not been uniformly successful in my hands. Csonka[4] has recently published a modification of the method to remedy this defect which involves the use of barium chloride solution in the digestion mixture together with a specially constructed digestion and

extraction flask, by means of which he has obtained good results. A simple and at the same time a most effective way to obtain a perfectly colorless solution is to unite the chloroform extracts and shake them out once or twice with distilled water in a separatory funnel, and then dehydrate with sodium sulfate. The water quickly and completely removes all color from the chloroform.

While the Autenrieth-Funk method has been used throughout as the simplest and most accurate of the colorimetric methods, the method recently described by Bloor[3] has been run parallel with it in several analyses. Bloor's statement that his method gives considerably higher results than the Autenrieth-Funk method has been entirely confirmed, but in the light of what is to follow, it appears that to whatever cause this increase is due, it is not due to cholesterol itself. A possible cause for a part, at least, of the excess value may be found in the slight brownish color invariably present in the chloroform solution of extracts prepared by Bloor's method. The alcohol-ether extraction method itself is most excellent, and has been used with good results in gravimetric determinations, as will appear later, but when an aliquot of this is evaporated, no matter how carefully one attempts to avoid heating the residue after it is dry (which Bloor claims to be the cause of the brown color), and even when it is evaporated before a fan at room temperature, there is still a slight brown tinge to the chloroform solution of the residue. This may be, however, only one factor which raises the value, and other things may play even a more important part.

In order to check the error in the digitonin determination, a few analyses were made on Merck's pure cholesterol. The cholesterol was dissolved in alcohol, brought to boiling, and a 1 per cent solution of digitonin in 90 per cent alcohol added, using 25 per cent in excess of the calculated quantity. This proportion of excess of the precipitant has been used in all the work. After precipitation, two procedures are available. In either case several hours are allowed for precipitation to become complete. In this laboratory it has always been placed in the ice box over night. Then, if one follows the original method of Windaus,[2] it is filtered on a Gooch filter, washed with alcohol and ether, dried in the air bath, and weighed. Since the compound is known to be slightly

soluble in alcohol, Fraser and Gardner[6] have modified the Windaus technique by washing with ether followed by boiling water, thus avoiding the use of alcohol in washing, and they use either a Gooch filter or tared filter papers in which the tared paper is washed with ether and water parallel with the paper containing the precipitate. This latter method has several advantages over the Windaus technique. In the first place, once the precipitate becomes firmly packed down on a Gooch filter, subsequent filtration and washing is very slow indeed; moreover, by the use of filter papers, several determinations can be carried out at the same time. The avoidance of alcohol washings probably obviates a slight loss in weight, since the precipitate is somewhat soluble in it. On the other hand, the washing with boiling water is probably a much more effective means of removing excess digitonin from the precipitate. Digitonin in a 1 per cent solution quite readily crystallizes out, and will not again go into solution until the mixture is heated up to about 60°. Therefore, if such a crystallizing out of digitonin should occur in a determination which was subsequently washed with cold alcohol and ether, it would almost certainly not dissolve, and the result would of course be too high. The procedure of Fraser and Gardner has, therefore, been followed in all practical determinations made in this laboratory. The weight of the precipitate is multiplied by the calculated factor 0.2431 to obtain the weight of cholesterol present rather than the approximate factor 0.25 recommended by Windaus to cover loss of the compound in washing.

In four analyses the results were as follows:

Cholesterol.		Remarks.
Taken.	Recovered.	
gm.	*gm.*	
0.0200	0.0204	Filtered on tared filter papers and washed with ether and boiling water.
0.0200	0.0205	
0.0200	0.0198	Filtered on a Gooch filter and washed with alcohol and ether.
0.0200	0.0201	

Since, then, accurate results are to be obtained by either of the two procedures as long as pure cholesterol is used to begin with,

[6] Fraser, M. T., and Gardner, J. A., *Proc. Roy. Soc., Series B*, 1909, lxxxi, 239.

what is the explanation of the marked discrepancy in analyses of blood? The greatest possibility for error, of course, lies in the preliminary extraction method. A complete extraction cannot be attained by extracting blood directly with an immiscible solvent, because of the formation of emulsions, and also because cholesterol apparently forms some kind of a physical or loose chemical combination with the proteins, from which it cannot be removed by such solvents as ether and chloroform. If the first difficulty is overcome by drying the blood with anhydrous sodium sulfate and grinding, and then extracting with ether in a Soxhlet, still the removal is incomplete.

In the Autenrieth-Funk method the completeness of the extraction depends on the preliminary breaking up of the protein molecules themselves by digesting on a water bath for 2 hours with 25 per cent potassium hydroxide in the proportion of about one part of blood to ten of alkali. This mixture will then give up the cholesterol to either chloroform or ether. Of course in such a process, the esters of cholesterol are saponified, so that it would be useless to extract in this way for the digitonin method, in which the main point is to determine separately the free and combined cholesterol.

If blood is added directly to alcohol, and the precipitate thus obtained extracted with boiling alcohol or by ether, or if blood is run slowly into a mixture of three parts alcohol to one of ether, using three parts of blood to one hundred of solvent, and the whole then brought to a boil on a water bath (Bloor's method) the extraction will be found to be practically complete, as shown by qualitative tests of the protein residue after filtration.

The following experiments will serve to illustrate the foregoing points:

Two 20 cc. portions of dog blood were dried with sodium sulfate and finely ground. These were then extracted in a Soxhlet apparatus for 1 and 5 days respectively, and the cholesterol was determined in the extracts by the digitonin method. Duplicate portions of 2.5 cc. each were analyzed colorimetrically by the method of Autenrieth and Funk.

	per cent
Colorimetric values, A	0.289
B	0.287
Gravimetric values, 1 day	0.125
5 days	0.150

Several specimens of blood were analyzed both colorimetrically using the method of Autenrieth and Funk, and in some instances also that of Bloor, and gravimetrically after precipitating the sample (10 cc.) with alcohol, and extracting the precipitate 24 hours with boiling alcohol in a continuous extraction apparatus, then concentrating the combined alcohol extracts, diluting with water, and extracting with ether.

Following are a few typical analyses:

Source.	Autenrieth-Funk.	Bloor.	Digitonin.
	per cent	*per cent*	*per cent*
Dog	0.185		0.151
"	0.231	0.267	0.169
Horse	0.151	0.186	0.100

The protein residues, after extraction with boiling alcohol, were digested for 2 hours with 100 cc. of 25 per cent potassium hydroxide, cooled, and extracted with 50 cc. of chloroform, the chloroform concentrated to about 5 cc., and to this 2 cc. of acetic anhydride and 0.1 cc. of concentrated sulfuric acid were added. Only a very faint yellowish green color was obtained, showing practically a complete extraction. The color obtained was about equal to that produced by about 0.0001 gm. of cholesterol in the same volume.

The above figures illustrate the lower results obtained by the digitonin method, even when extraction was probably complete. It did not, however, seem quite fair to make comparisons which would leave room for question on this score. Several experiments, of which the following are types, were carried out.

12 cc. of sheep blood were digested for 2 hours with 100 cc. of 25 per cent potassium hydroxide, and the resulting liquid was extracted once with 250 cc. of chloroform and three successive times with 125 cc., shaking vigorously for 5 minutes each time. (This proportion of chloroform to solution and time of shaking are recommended by Autenrieth and Funk.) The combined extracts were shaken out with two small portions of water to decolorize, and then dried over sodium sulfate. After concentrating, the residue was transferred to a 100 cc. volumetric flask, filled to the mark, and 16.7 cc. (= 2 cc. of blood) were removed for colorimetric analysis. The remainder (= 10 cc. of blood) was evaporated to dryness, taken up in alcohol, and precipitated by digitonin. A second portion of blood was analyzed colorimetrically by Bloor's method.

	per cent
Colorimetric result	0.136
Gravimetric "	0.114
Colorimetric " (Bloor)	0.182

The experiment was repeated with 12 gm. of sheep liver, the alkaline mixture being autoclaved 1 hour at 15 pounds' pressure, instead of being boiled for 2 hours.

	per cent
Colorimetric result	0.409
Gravimetric "	0.374

Dog blood digested as in the case of the sheep blood gave the following figures:

	per cent
Colorimetric result	0.182
Gravimetric "	0.152
Colorimetric " (Bloor)	0.216

Bloor states, in accounting for his higher results, that probably a portion of the cholesterol is destroyed by the long continued boiling with strong alkali in the Autenrieth-Funk method. This is a reasonable assumption, and, if true, would equally well explain the lower gravimetric figures. In order to bring about extraction and saponification without the use of such strong alkali the following experiments were done:

Two samples of dog blood were obtained, and 15 cc. portions were extracted by the method of Bloor, using, for each, 500 cc. of a mixture of three parts alcohol and one part ether, and adding the blood slowly to about 400 cc., bringing to a boil, cooling, and making up to the mark in a 500 cc. volumetric flask. The mixture was filtered by suction and the precipitated protein sucked as dry as possible, but not washed. The filtrate was made up to 500 cc. A 5 cc. portion was removed for colorimetric determination. The remaining 495 cc. were in each case evaporated down to 15 to 20 cc., taken up in water, and thoroughly extracted with four portions of ether. The aqueous residue after ether extraction was evaporated to dryness, 25 per cent potassium hydroxide added, and heated for a half hour on the water bath, then extracted with chloroform. The chloroform extract concentrated to about 5 cc. gave only a trace of yellowish color with acetic anhydride and sulfuric acid; hence extraction was complete. The ether extracts were united, concentrated, and made up to 100 cc. A portion equivalent to 2 cc. of the blood was removed for colorimetric analysis, the remaining ether evaporated off, and the residue (= 13 cc. blood) saponified for 2 hours with 50 cc. 0.5 N alcoholic potash under a return condenser. The greater part of the alcohol was then evaporated off, the residue taken up in water, and thoroughly extracted with ether. The aqueous residue again showed no cholesterol when tested as described above. The ether extracts were concentrated, made to 100 cc., a portion equivalent to 2 cc. of blood was removed for colorimetric analysis, a further portion equivalent to 1 cc. removed and discarded, the remaining ether

extract, equivalent to 10 cc. of blood, evaporated to dryness, and the residue taken up in alcohol and precipitated with digitonin.

Determinations were also made on the blood by the Autenrieth-Funk method.

The results were as follows:

	I.	II.
	per cent	*per cent*
Colorimetric result (Bloor's method, 5 cc. of extract)	0.228	0.293
" " ether extract, unsaponified	0.176	0.233
" " " " saponified	0.174	0.237
Gravimetric "	0.159	0.208
Colorimetric " Autenrieth-Funk method	0.197	0.239

With the exception of the figure obtained by the Autenrieth-Funk analysis of Sample I, which is apparently too high, the figures compare very well with those of the preceding experiments. The striking decrease in the colorimetric value produced only by extracting the concentrated alcohol-ether solution with ether, and thus eliminating some brownish pigment which always is present in the latter, is shown well in both sets of analyses, and there is apparently no further loss after saponification. All of the material, however, which contributes to the green color is evidently not cholesterol, for it cannot be precipitated by digitonin.

There was still the chance that the 2 hours' saponification with alcoholic potash so altered some of the cholesterol that, while it still gave the color reaction it failed to precipitate with digitonin.

To see if this were the case, two samples of 10 cc. each of horse blood were extracted by Bloor's method, and to the alcohol used in one case 0.02 gm. of pure cholesterol was added. These two extracts were saponified and precipitated as above, the resulting amounts of cholesterol obtained being respectively 0.0076 and 0.0274 gm. There was consequently 0.0198 gm. of the cholesterol recovered in spite of the 2 hours' saponification. This made it pretty certain that there was little change in the cholesterol by the alkali, for by mass action more cholesterol should have been changed in the second case than in the first, if a change took place, and a greater loss would have resulted. But to avoid any error on this point, the effect of saponification with 0.5 N alcoholic potash was tried on pure cholesterol. Three portions of 0.0100 gm. of cholesterol in 10 cc. of alcohol were pre-

pared. One portion was immediately precipitated with digitonin. The second was saponified for 1 hour, and the third for 2 hours, then extracted and precipitated. The amounts recovered were respectively 0.00958, 0.00948, and 0.00943 gm., so that there was practically no loss after 2 hours' saponification.

In a number of determinations the ether washings from the digitonin-cholesterol precipitate were extracted once or twice with water to remove the excess digitonin, evaporated to dryness, taken up in 5 cc. of chloroform and acetic anhydride, and sulfuric acid was added. After a short time an unmistakable olive green color always appeared, having, however, a brownish cast. This was equal in intensity perhaps to that which would be produced by 0.002 gm. of cholesterol, and, since the precipitate of cholesterol in the average experiment was usually about 0.015 gm., it amounts to considerably more than 10 per cent of the total color which could have been produced by the material before precipitation. The green color produced by these filtrates faded out rather more quickly than the cholesterol color, and left a yellowish brown solution. In one case, where the residue was treated with glacial acetic acid and a few drops of concentrated sulfuric acid, a slightly reddish color was produced (oxycholesterol reaction?—see below).

DISCUSSION.

It is apparent from the foregoing that the values obtained by the colorimetric method represent some additional substance or substances beside cholesterol itself. That substances closely related to cholesterol exist in the blood normally is shown by the work of Lifschütz.[7] So called "oxycholesterol," a substance which may be prepared artificially from cholesterol by oxidation with potassium permanganate, ferric chloride, or benzoyl peroxide in glacial acetic acid solution, and characterized chemically by the production of a reddish violet color passing into green, with definite spectrum lines upon the addition of sulfuric acid to its acetic acid solution, has been isolated by him from the blood, and a method worked out for its quantitative determination.

[7] Lifschütz, J., *Z. physiol. Chem.*, 1906-07, l, 437; 1907, liii, 140; 1908, lviii, 175.

There is some question as to the properties of these oxidation products, of which there are two or three which can be recognized spectroscopically. Lifschütz[8] states that they will give the Liebermann-Burchard color reaction, and also that they will precipitate with digitonin from alcohol solutions, while Schreiber[9] denies the latter point. Since much of this oxycholesterol work remains as yet unconfirmed it is scarcely desirable to speculate about the exact part which is played by these derivatives in the results obtained in "cholesterol" determinations. Certain it is that they, or some related substances present in the blood, add to the green color of the Liebermann-Burchard test, but fail to precipitate with digitonin. It is quite possible that there may be a partial precipitation of some of this material by the digitonin, and that the gravimetric results themselves are somewhat in excess of the actual amount of true cholesterol present. No attempt has been made to determine whether such a condition exists or not. In one experiment it was shown that there was no wide divergence from the theoretical formula for the precipitate by boiling a sample weighing 0.0111 gm., which had been obtained from dog blood, with 0.5 N alcoholic potash for 2 hours, which gradually dissociates the compound and destroys the digitonin. After a thorough ether extraction of the residue, there could be recognized colorimetrically 0.0026 gm. of cholesterol, while the amount present theoretically was 0.0027 gm. Of course the presence of any oxycholesterol-digitonin compound is not disproved by such an experiment, since the formulas would be closely related, cholesterol being $C_{27}H_{44}O$ and oxycholesterol $C_{27}H_{44}O_2$. For the present, however, it seems warranted to look upon the digitonin determinations of cholesterol in blood as being very nearly correct values when care is taken to insure complete extraction. Values obtained by colorimetric methods should be considered as representing cholesterol plus some more or less closely related substance or substances, very likely of the nature of oxidation products.

[8] Lifschütz, *Münch. med. Woch.*, 1913, lx, 1549, 2346.
[9] Schreiber, E., *Münch. med. Woch.*, 1913, lx, 2001.

CONCLUSIONS.

1. When applied to solutions of pure cholesterol either the colorimetric or the digitonin method of determination is accurate.

2. Ether extraction of dried blood, even when prolonged, is not complete, and recourse must be had to hot alcohol or alcohol and ether.

3. Colorimetric analyses of blood give results too high for true cholesterol, because they include other ether- and chloroform-soluble substances, whereas digitonin determinations are more nearly correct.

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LIMITATIONS TO THE CULTIVATION OF MAMMALIAN TISSUES IN VITRO

R. A. LAMBERT, M.D.

(From the Pathological Laboratory of the Presbyterian Hospital, New York)

A number of enthusiastic papers on the subject of tissue cultures, for some of which the writer was responsible, have undoubtedly give rise to erroneous impressions as to the possibilities of the method, especially regarding the variety of mammalian tissues which may be cultivated. It was, I believe, with the idea of correcting certain of these misconceptions that the President of the Society suggested the present report of my experience with the method.

I should like first of all to present a classification of tissues based on their behavior in vitro. Afterwards the factors which may possibly be responsible for failure in the propagation of certain of the tissues will be briefly considered.

Our experience has suggested that tissues may be grouped as follows:

1. Tissues showing no cell multiplication or migration in cultures. Microscopic sections indicate that the cells in the tissue fragments survive for a time. Examples are: highly specialized cells, such as liver and kidney epithelium, and ganglion cells; also those of human malignant tumors.

2. Tissues showing no multiplication but active wandering. The cells die and undergo disintegration after a few days' activity. Examples are: leukocytes, mononuclear wandering cells of spleen, lymph glands and bone marrow and epithelium of skin and cornea.

3. Tissues showing both active multiplication and migration which cease after a short time in spite of efforts directed toward continued propagation. Examples: malignant tumors of rats, mice, and fowl.

4. Tissues showing active multiplication and migra-

tion with tendency to progressive increase of growth in subcultures. Example: connective tissue cells of all species.

It is clearly important to know the normal behavior of various tissues in culture, as otherwise erroneous interpretations of experiments are apt to be made. Spleen, for example, was used by Carrel and Burrows,[1] in studying the influence of physical changes in the plasma medium on cell activity. The apparent stimulating effect observed has been referred to as an "activation of growth." Since the cells which migrate during the first two or three days in spleen cultures, are leukocytes and mononuclear wandering cells which show no evidence of multiplication, it is obvious that there could have been no true stimulation of growth.

Why certain tissues fail to grow and others exhibit only a brief period of growth in vitro is a question not readily answered.

Examining the list of tissues given above it is to be noted first that many specialized epithelial cells show only a brief survival. This is not surprising in view of the recognized susceptibility of such cells in the body to mechanical and toxic injuries, or even to mere disturbances in nutrition. Indeed it is rather remarkable that such sensitive elements as ganglion cells should survive for any considerable period. Also the temporary activity of leukocytes and related cells of the spleen, lymph glands and bone marrow, is not to be wondered at, since the life of these cells in the body is probably very short, and they do not reproduce themselves even in vivo.

On the other hand, it is surprising that the cells of malignant tumors of rats and mice should show only a brief period of activity in contrast to the prolonged, apparently unlimited, growth of connective tissue cells of those species; also that the cells of human malignant tumors cannot be cultivated by methods which allow the growth of normal cells and benign tumor cells.[2]

In seeking the explanation for these differences in the behavior of tissues in vitro several factors may be considered: mechanical injury, accumulation of toxic metabolic products, presence of inhibiting antibodies, and nutritional disturbances.

Mechanical injury. This factor is certainly important in

determining success in the cultivation of any tissue, as small experience with the method will show. But much evidence could be submitted, especially regarding the rat and mouse tumors, to prove that tumor cells are not particularly susceptible to traumatic injury. Besides, such injuries would explain only the failure of the cells to grow in primary cultures and would not account for the early cessation of growth observed.

Accumulation of metabolic products. In tissue cultures, put up according to the method in common use, there is no mechanism for the removal of waste products, or for the continuous supply of fresh nutritive medium. It is conceivable, therefore, that the accumulation of the products of metabolism may exert an inhibiting action on the growth of very active cells. In order to eliminate this factor as far as possible, early transfers have been made and the tissue fragments have been washed for varying periods in salt solution or hypotonic Ringer's solution as recommended by Carrel[3] and his co-workers who attributed their success in the prolonged cultivation of embryonic connective tissue largely to this procedure. We did not find that this treatment changed our results. It may be stated in this connection also, that, in the cultivation of adult connective tissue, an increasingly active growth was observed when the tissue fragments were transferred to fresh medium without washing.

It has been concluded, therefore, that the factor of inhibition by metabolic products does not serve to explain the failure of certain tissues to grow, or the limited growth of others.

Presence of inhibiting antibodies. While it has been established by various methods that antibodies for tumor cells do not exist in the circulating blood of rats and mice, it has been more difficult to settle this question for human tumors. Freund and Kaminer[4] have reported the discovery in human blood serum of two substances affecting tumor growth; one in the blood of patients suffering from cancer which exerts a protective influence on the cancer cells; the other in non-cancerous individuals which is lytic for cancer cells as demonstrated by test-tube experiments. While the experiments of these authors have not received con-

firmation by competent investigators, their work cannot be entirely disregarded.

In earlier experiments we used, as a matter of convenience, blood plasma from several normal individuals as a culture medium for human tumors. The negative results obtained and the work of Freund and Kaminer noted above, suggested that the plasma from the tumor-bearing individual might prove a better medium. It also occurred to the writer to see whether the presence of isoantibodies, agglutinins and hemolysins, influenced the growth in vitro of either tumor or normal tissue. This study has shown first that human carcinoma and sarcoma cells are not readily cultivated in vitro even in plasma taken from the individual carrying the tumor, while normal human connective tissue cells and benign tumor cells (mixed tumor of parotid) grow as well in homologous as in autogenous plasma. Furthermore the presence of isoantibodies is without demonstrable effect.

Nutritional disturbances. Before discussing this factor it may be well to consider the question advanced recently by Burrows as to whether cells growing in vitro can metabolize the food stuffs in the plasma medium. Burrows has expressed the view that the cells do not get nutriment from the medium at all. He assumes that they live on food carried over in the cell cytoplasm, a condition analogous to that seen in many developing ova.

In my opinion, a number of observations make this view untenable. In the first place, it is obviously impossible that a sufficient quantity of nutriment could be present in the cell cytoplasm to maintain active multiplication and wandering for any considerable period, whereas we know that active life in vitro, in the case of connective tissue cells at least, can be maintained for weeks, months or possibly years (Carrel). In the second place, if the cells did not take up nutriment from the medium succeeding generations should show a progressive diminution in size; but careful observation proves that this is not the case. Thirdly, the growth of cells in salt solution or greatly diluted plasma, where the food supply is markedly reduced, is of short duration and the cells of the second and third generation do become smaller. They show also very little fat accumulation as the result of the reduction in

the quantity of this food stuff in the medium.[5] It therefore must be admitted, I think, that cells do take up nourishment from the plasma in which they are growing.

If the failure of certain cells to grow is to be attributed to the food supply, the trouble must lie either in an inadequate supply or in the absence of certain specific food stuffs. That the quantity of nutriment is adequate is indicated by dilution experiments with those tissues which can be cultivated. Practically all such tissues will grow in plasma diluted with equal parts, or more, of salt solution without any effect on the rate or duration of growth.

Regarding the second possibility, namely, the absence of special food stuffs, it may be suggested that connective tissue cells can be cultivated for long periods in plasma of foreign species.[6] This result is opposed to the idea of strict food specificity. However, in the hope of supplying some such necessary hypothetical substances, extracts from various organs including liver, spleen, thyroid, testis, malignant tumors, and adrenal, have been added to the culture medium. No effect from such additions was observed.

CONCLUSIONS

Connective tissue is the only mammalian tissue which can be cultivated in vitro for any considerable period.

The transplantable tumors of lower animals show very active growth for a short time only. The previous reports of prolonged cultivation of such tissues were probably due to confusion between tumor cells and connective tissue cells in the culture preparations.

Human malignant tumors have not been cultivated successfully by us, although human connective tissue cells and wandering cells show marked activity in cultures.

The limited growth of animal tumor cells and the negative results with human malignant tumors may be referable to several factors, mechanical injury, accumulation of metabolic products, presence of inhibiting antibodies, and nutritional disturbances; but my experience leads me to believe that no one of these factors is alone responsible.

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1. Carrel, A., and Burrows, M. T. *Jour. Exper. Med.*, 1911, xiii, 562.
2. Lambert, R. A. *Proc. Soc. Exper. Biol. and Med.*, 1916, xiii, 100.
3. Carrel, A. *Jour. Exper. Med.*, 1913, xviii, 287.
4. Freund and Kaminer. *Wien. klin. Wschr.*, 1912, xxv, 1698.
5. Lambert, R. A. *Jour. Exper. Med.*, 1914, xix, 398.
6. Lambert, R. A., and Hanes, F. M. *Jour. Exper. Med.*, 1911, xiv, 129.

THE CYTOLOGY OF THE EXUDATE IN THE EARLY STAGES OF EXPERIMENTAL PNEUMONIA

PLATES 15 AND 16

FRANK A. EVANS

From the Pathological Laboratory of the Presbyterian Hospital, New York City

On examination of sections from the consolidated lungs of pneumonia patients dying in the first few days of the disease it is readily appreciated that the cellular characteristics of the exudate in the early stages are not the same as in the almost exclusively polymorphonuclear exudate of the later stages. The early exudate is predominantly mononuclear in character, and is to be differentiated from that of the very late stages when again mononuclear cells are frequently numerous. In the later stages the mononuclear cells present are plasma cells and desquamated alveolar epithelial cells. The mononuclears seen in the early exudate are not, however, so readily classified. Several distinct types must be recognized (Fig. 1), and each type is constant in its occurrence. It has been our aim to identify some of these cells in the pneumonic processes experimentally induced in rabbits, and in the processes early in 3 cases of human pneumonia.

The pneumonic process was induced in each rabbit by intrabronchial injection of 8 c.c. of an 18-hour neutral-broth culture of the organism after the method of Meltzer and Lamar.[1] The organisms injected were strains from Pneumococcus Group I and Group IV, an attenuated strain of pneumococcus of undetermined group, a hemolytic streptococcus, and a streptococcus isolated from the mouth of a normal individual. The exudate in reaction to intrabronchial injection of 33% egg yolk in neutral broth was also studied.

The lesions were examined at periods varying from 8 to 36 hours. In some cases the gross distribution of the process was fairly regular, embracing an entire lobe or all the lobes of one side, tho it was never entirely homogeneous as in human lobar pneumonia; in other cases the distribution was patchy. The even distribution was commonly encountered after pneumococcus injection, and the patchy after the injection of a streptococcus, but this was not constant. In a number of the rabbits the alveolar walls were infiltrated, the infiltrating cells being of the same character as those in the exudate (Fig. 1). The cytology

[1] Jour. Exper. Med., 1912, 15, p. 133

of the early exudates in the three human cases of pneumococcal pneumonia, coming to autopsy on the 3rd, 4th, and 5th days respectively, was the same as that of the experimental pneumonia in rabbits (Fig. 4).

Except for slight variations in the relative proportions in which the cells were present, depending to some extent on the organisms, but more particularly on the age of the exudate, the cytology was the same in all the preparations. Typical small lymphocytes of the blood were always present in small numbers and were easily identified. Even in the very early exudate some polymorphonuclears were constantly seen, perhaps only one to an alveolus in some places, but to the exclusion of all other types in other alveoli in the same section. A few very large mononuclear cells also were frequently encountered which, by reason of their morphology and staining properties, could be definitely classified as epithelial cells in various stages of degeneration. In addition to these there were other mononuclears which were the predominating mononuclear cells, and except in those alveoli where the polymorphonuclears outnumbered them, they were the predominating cells of the exudate. These cells, always much larger than the polymorphonuclears, yet smaller than the epithelial cells, fell into 2 groups, depending on whether or not they contained an oxydase ferment as demonstrated by the indophenolblue reaction[2] (Fig. 2). Other differential criteria were indefinite except that those containing an oxydase ferment commonly had a slightly or markedly indented nucleus with only a fair amount of chromatin and abundant homogeneous protoplasm that occasionally showed faintly a fine granulation, whereas those cells that did not contain an oxydase ferment had, as a rule, a round or oval nucleus often showing one or two flat surfaces and sometimes staining deeply, and abundant homogeneous protoplasm that never showed evidence of granulation. In form the typical cells of the first group strongly suggested the so-called transitional cells of the blood and were so considered by Pratt[3] in 1900. Their content of oxydase ferment makes this seem likely if myelocytes are not considered. Both myelocytes and so-called transitional cells give the oxydase reaction, and as Naegeli has pointed out[4] the differentiation between them in tissue sections is sometimes difficult. Certainly a few cells resembling myelocytes were to be seen. Myelocytes probably were present in small numbers, but, altho they must be considered, the morphology of most of the cells under discussion was not that of myelocytes and it

[2] Evans: Proc. New York Path. Soc., 1915, 15, p. 143.
[3] Johns Hopkins Hosp. Rep., 1900, 9, p. 265.
[4] Blutkrankheiten und Blutdiagnostic 1912, p. 193.

seems quite certain that they belonged to the so-called transitional-cell group. The other cells, those that did not give the oxydase reaction, were probably lymphoid in origin, and many of them resembled very much the pictures of polyblasts appearing in Maximow's articles.[5] They were not reticulo-endothelial cells, for tho such cells, vitally staining by carmin, are occasionally seen normally in the alveolar walls, in pneumonia in rabbits heavily stained by carmin these carmin cells were not seen taking part in the formation of the exudate. That they are closely related to the blood elements is further borne out by the fact that in pneumonia induced in rabbits poisoned by benzol,[6] whereby the leukocytes and leukopoietic tissue were destroyed, cells were seen in the exudate (Fig. 3). No plasma cells were seen in any of these preparations.

CONCLUSION

In the early exudate of pneumonia, altho many polymorphonuclears may be present, in many alveoli the cytology of the exudate is predominantly mononuclear in character. The mononuclear cells may be classified as follows: (1) a few typical small lymphocytes, (2) a few desquamated alveolar-wall epithelial cells, (3) relatively many oxydase-containing large mononuclears of the blood, belonging to the so-called transitional-cell group of Naegeli, and (4) almost as many non-xydase-containing large mononuclears of the blood or closely related forms.

EXPLANATION OF PLATES

A = small lymphocytes.
B = polymorphonuclears.
C = desquamated epithelial cells.
D = oxydase-containing large mononuclears.
E = non-oxydase-containing large mononuclears.

FIG. 1. Section of consolidated rabbit lung in which pneumonia has been induced by intrabronchial injection of Streptococcus hemolyticus. Animal killed 14 hours after inoculation. The cells infiltrating the alveolar walls are essentially the same as those in the alveolus.

FIG. 2. The indophenolblue reaction on a section of consolidated rabbit lung after pneumococcus injection. The nuclei are counterstained by 5% aqueous pyronin.

FIG. 3. Section of consolidated rabbit lung in which pneumococcal pneumonia has been induced after destruction of the leukopoietic tissue by administration of benzol.

FIG. 4. Section of consolidated human lung from a pneumococcal pneumonia, patient dying on the third day of the disease. This also represents accurately the condition in induced pneumococcal pneumonia in rabbits.

[5] Beitr. z. path. Anat. u. z. allg. Path., 1903, 34, p. 153; 1904, 35, p. 93; 1905, 38, p. 301.
[6] Selling: Ibid., 1911, 51, p. 576.

PLATE 1

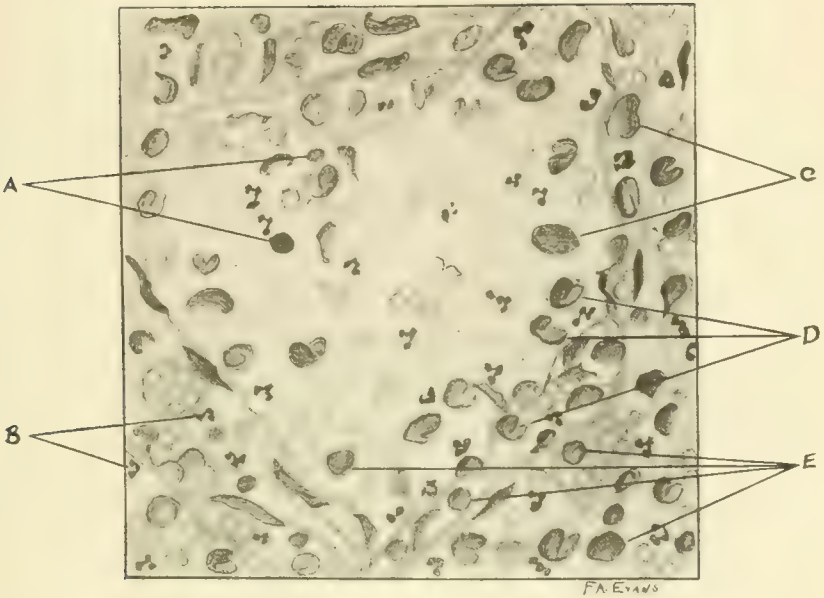

Figure 1

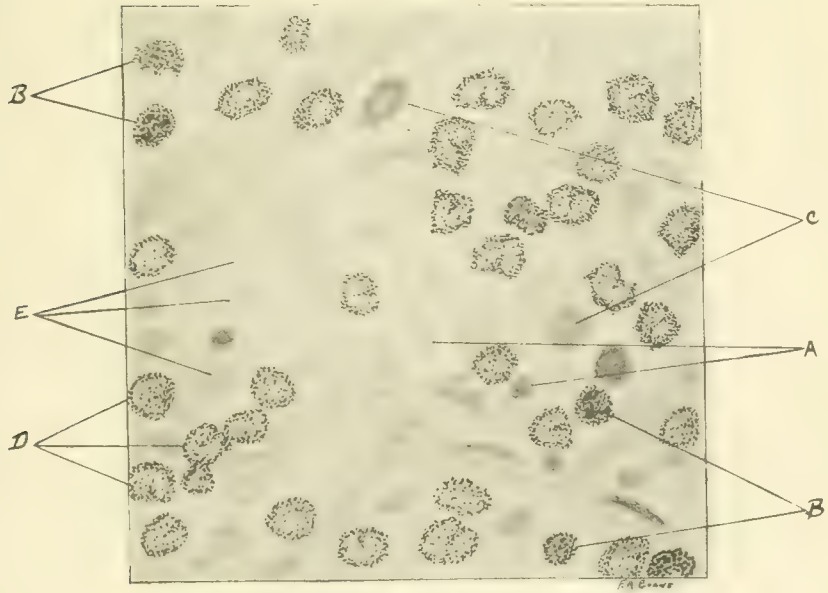

Figure 2

Figure 3

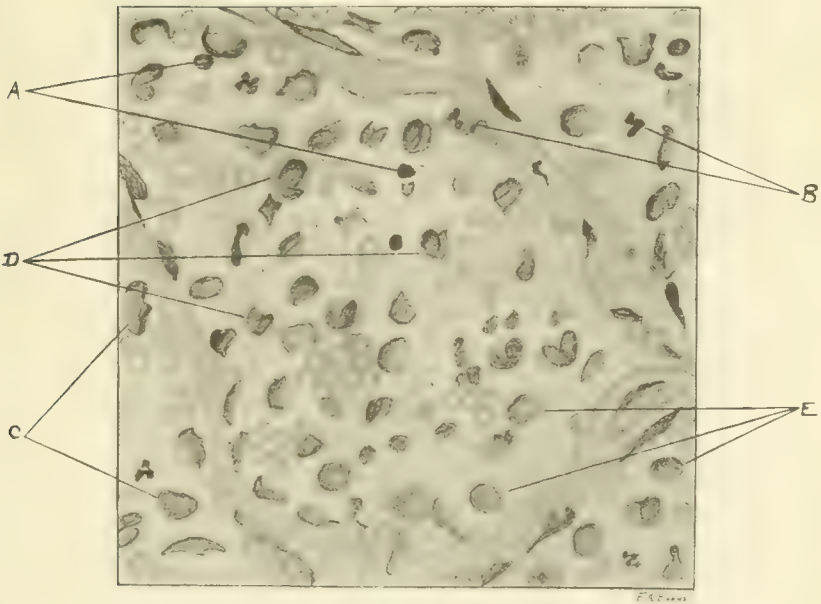

Figure 4

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ZOOPATHOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY ON THE DISEASES OF ANIMALS

VOLUME I, NUMBER 1

SOME NEW SPECIES OF PARASITIC TREMATODES OF MARINE FISHES

By G. A. MacCallum, M. D.
Department of Pathology
COLUMBIA UNIVERSITY, NEW YORK
Pathologist
NEW YORK AQUARIUM

PUBLISHED BY THE SOCIETY
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Each author is responsible for the scientific accuracy
and the proof reading of his contribution.

ERRATA

pluroxittellum should read *pluroxitellum* wherever it occurs.
Merlucius should read *Merluccius* wherever it occurs.
merlucii should read *merluccii* wherever it occurs.
hematobium should read *hematobia* wherever it occurs.
On page 28 the width of Chitinous Cirrus should read .015.

FIGURES

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Fig. 1. *Synbothrium lintoni* ..... face 6

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ABBREVIATIONS

Abbreviations in explanation of plates:

b.s.—buccal sucker
c.s.—caudal sucker
c.p.—cirrus pouch
cl.—cloaca
c.—cirrus
b.ej.—bulbus ejaculatorius
d.ej.—ductus ejaculatorius
e.—eye
ex.p.—execretory pore
ex.v.—execretory vessels
g.p.—genital pore
g.a.—genital atrium
h.—hook.
i.—intestine
m.—mouth
m.b.—muscular bands
m.g.—mucus glands
met.—metraterm
o.s.—oral sucker
ov.—ovary
oot.—ootype
oe.—oesophagus
ov.d.—oviduct
ph.—pharynx
p.p.—pars prostatica
pr.—prostate
r.p.—reservoir prostatica
p.s.—penis shell
s.g.—shell gland
s.v.—seminal vesicle
s.r.—seminal reservoir
s.p.—sensory papillae
t.—testis
t.a.—tactile area
u.—uterus
u.g.—unicellular glands
v.d.—vas deferens
va.—vagina
vit.—vitellaria
v.s.—ventral sucker
v.r.—vitelline reservoir
w.v.s.—water vascular system
y.d.—yolk duct
y.r.—yolk reservoir

SOME NEW SPECIES OF PARASITIC TREMATODES OF MARINE FISHES

By G. A. MacCallum, M. D.
Department of Pathology, Columbia University, New York.
Pathologist to the New York Aquarium.

Introduction

In presenting the following papers, it is desired to say that the forms from marine biological life which are herein described were studied in material from the New York Aquarium and the United States Fish Commission Laboratory at Wood's Hole. However imperfect the descriptions may be, the plates that are used are from sketches made by the author from life and from mounted specimens in our collections, and we believe them to be as true to nature as it is possible to make them. Other than the outlines of the form of the worm, the studies are of the reproductive organs, inasmuch as there is little else to show in the bodies of these hermaphroditic creatures, whose whole object in life seems to be the reproduction of their kind.

From the material above described, the able artist, Mr. Feinberg, has shown in a very realistic way all that is necessary to give the reader a good idea of the anatomy of the specimen. We desire to say also that, with the exception of possibly two, all of the worms as far as we know are new to science.

It may perhaps be interesting to some to describe briefly the process of preparing these specimens for study. The following technique is generally followed: The worms are first washed in sea water, as fresh water is rapidly absorbed by the body, which becomes swollen and stiff. After the washing they are put under slight pressure and killed in a hot solution of bichloride of mercury, then washed again, after which they are stained with acid carmine, and next passed through several alcohols, and cleared in cedar oil or creosote and xylol, and mounted in balsam. This process for trematodes is one of the most satisfactory plans to bring into view the various anatomical structures, and differentiate them so that they may be seen easily

It must be borne in mind that all these forms are parasitic, and many of them seriously endanger the welfare of their host, hence in many cases it would be of economic value to study their habits of life and life history. This, however, has generally proved almost impossible, and only in a few cases has it been satisfactorily done, and these have had to serve for the numerous cases by analogy for those in which the life history has had to be guessed at. We know that they are produced from the egg; but how, in the open sea, these microscopical eggs find

their way into the unusual habitats of the host, is largely speculative. It is known, of course, that the egg under favorable circumstances may develop into a free swimming miracidium or ciliated larva which by chance may meet with a fish or other host, when a lodgement may be made, and when it may by developed, for it cannot by developed in any host. The chances are millions against such a thing occurring, so that nature provides such creatures with myriads of eggs on the chance that a few at least will find a favorable host. Of course all this does not apply to those parasites whose eggs are laid and fastened directly on the tissues of the host, where they are hatched.

As is so well known, bacteria and bacilli are the principal parasites in the human diseases which have of late years received much attention from scientists with such good results. These same microscopic parasites attack fish in various ways, and in the future will probably receive the attention they deserve.

I wish here to thank Dr. Townsend, Director of the Aquarium of New York, for his unselfish kindness in forwarding our work in every way. Also the same is due the officials of the Fish Commission at Wood's Hole for their unremitting kindness in furnishing material.

Dasybatus pastinacus (Walbaum, 1793).

Sting Ray or Stingaree.

It is highly probable that this fish is infested with more parasites than any other known fish. Twenty-seven different forms, not including copepods, are already credited to it and almost any examination of new material develops some new worm.

At Wood's Hole of late seasons, several specimens have been taken of large size from ten to twelve feet in length, including the tail, which is nearly twice the length of the body. These were generally brought to the Laboratory of the United States Fish Commission and examined for parasites, etc. During the last two or three seasons ectoparasites or external parasites have occupied our attention chiefly and it is proposed in the following paper to describe a few of the hitherto undescribed forms which have been met with in examining the sting ray.

Synbothrium lintoni, nov. sp.

(Figs. 1 and 1A)

There does not seem to have been much written upon this cestode, and very few species described.

Diesing seems to have been the first in 1850 to record a worm which he found in a Brazilian fish t. h. *Pristis perottetii* which he called *Syndesmobothrium* in 1854 but which he afterwards renamed *Synbothrium fragile* as the type 1850a. There have been a few au-

Fig. 1

Fig. 1-A

FIG. 1. *SYNBOTHRIUM TINTONI* FIG. 1-A. RIPE PROGLOTTIS, *S. TINTONI*

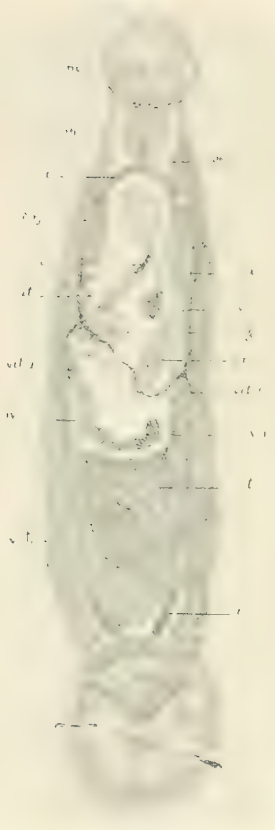

Fig. 2

Fig. 2-A

Fig. 2-B

Fig. 2. *MONOCOTYLE DASYBELIS*. Fig. 2-A. SIDE VIEW OF *M. DASYBELIS*. Fig. 2-B. EGG OF *M. DASYBELIS*.

thors since, as Burm. Goldb. Lint. Rail, Scudder, Zsch., etc., who have described its occurrence in a few species of fish, but it does not appear to be a very common parasite, consequently there have been very few species of it named; in fact, *S. fragile*, Diessing 1850 and *S. filicolle* Lint. 1889 seem to be the only ones.

Linton appears to have found *S. filicolle* in a number of fishes, but *S. fragile* in only one instance in 1901. In 1905, however, he came across *Synbothria* sp. in four different species of fish, and these he did not name.

These worms have, I believe, almost always been found in a larval state, encysted in the viscera or peritoneum of the host, so that one cannot always be certain of the species. All of the Synbothrium worms which I have found thus far, except *S. lintoni*, have been encysted in the abdomen of the following fishes: *Epinephelus morio*, *Oxyurus chrysurus*, and *Pogonias cromis*, and were of two species, one *S. filicolle* and the other not as yet named. In these cases they were enclosed in an outer cyst which was colored blackish, and within this the true white blastocyst in which the young worm may be seen coiled, so transparent indeed that the hooks might be seen. These cysts are never plentiful as far as my experience goes, at least I have never found more than three or four in the same host, and often only one.

However, on August 1, 1914, while working at the United States Fish Commission Laboratory at Wood's Hole a large sting ray *Dasybatus pastinacus* fell to my share, and, while examining the contents of the spiral valve, I found among the denizens of that interesting structure, four cestodes which were unknown to me. They proved to be adult, and clung to the mucous membrane with their proboscidae. They were with little difficulty freed from their attachment, and proved to be *Synbothria* of a new species. They were quite large, varying in length from 25 mm. to 65 mm. and 2 mm. in width, and some showed ripe proglottides containing eggs. They also showed that other segments had been thrown off, so that really one could not say what the original length of the worm may have been. The head as a whole is quite different in shape from any other *Synbothrium* I have seen, being in the gross much the shape of the head of a Scotch thistle in bloom, set upon a narrow neck, which is followed by a strobila of gradually maturing proglottides.

The bothria are much of the same form as those of other members of the genus. The four bothria occupying the four separate angles and from each of which forming a pedicel as it were a proboscis protrudes form the extreme point. The bothrium in each case lies beside the opening of the proboscis sheath. These prominent portions of the head are exceedingly mobile, being capable apparently of moving in any direction. The proboscides are long and strong, being covered with hooklets which are arranged more or less diagonally around the base or core in several rows, probably ten or twelve. The hooks

appear to be of different shapes and character—each variety is placed in a row by itself which holds throughout the length of the proboscis. One row situated along the inner or concave surface of the proboscis is composed of short strong and much curved hooks and attached to a smooth surface, while the other rows are composed of long slender slightly bent hooks, with the points all directed backwards towards the head and fixed on a surface which is thickly covered with small hooklets or bristles, which give the impression that some of them are young or undeveloped hooks. Under a higher lens power, one can count five rows of the larger hooks to the field, so that there are about ten or twelve rows around the core; they are all composed of semitransparent chitinous material. The bothria and indeed the whole anterior end of the worm under the cuticle is seen to be composed of numerous delicate muscular fibres interlacing in all directions transversely, obliquely and longitudinally, thus giving the head its power of motility. The tubular proboscis sheaths or canals terminate posteriorly in four contractile muscular bulbs which control the protrusion or withdrawal of the proboscidae. The striations in the strobila do not appear at once in the neck, but they show indistinctly to very near the head. The water vascular system is very evident in the form of a canal along each side of the worm throughout its length.

The ripe proglottides are about 2 mm. long and 1.50 mm. wide, and the cloaca, a deep notch with prominent lips, appears on the edge of each segment at the junction of the posterior third with the anterior two-thirds. The cirrus is often seen protuding outside of the anterior edge of the cloaca and it is there so large and bulbous that it seems to block up the outlet of the uterus. The cloaca may appear on the same side of the worm for three or four segments in succession. They are not regularly placed. The eggs are yellow, oval or rounded, not very numerous, and those measured were .040 mm. long by .020 mm. wide.

The segments are largely filled with testes which appear as more or less straight tubes, extending nearly from one end to the other, and parallel to one another. The vas deferens arises from these and terminates in the cirrus. The vitellarium cannot be made out in the material at hand.

Measurements of *Synbothrium lintoni*, nov. sp.

Length	65.00	mm.
Width	2.00	mm.
Head—length	3.40	mm.
Head—width	1.80	mm.
Neck or first segment	.60	mm.
Proglottid—length	2.00	mm.
Proglottid—width	1.50	mm.
Eggs—length	.04	mm.
Eggs—width	.02	mm.

Fig. 3

Fig. 4

Fig. 4-A

FIG. 3. *MONOCOTYLE DISYBATHIS MINIMUS.* FIG. 4. *MERIZOCOTYLE DISYBATHIS* FIG. 4-A. GENITAL SCHEME OF *M. DISYBATHIS.*

Monocotyle dasybatis, nov. sp.

(Figs. 2 and 2A and B)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Mont.).
Family *Monocotylidae* (Tasch.).

On July 17, 1915, five of the above worms were found in the gills of a large female sting ray which measured seven feet nine inches in length by three feet three inches in width. The worms measured seven to eight mm. in length by 1.80 mm. in width and were found in the profuse thick mucus which makes an examination of the gills of this fish difficult. It is true that the worm found in the gills of *Myliobatis aquila* Dumeril, one of the eagle rays in European waters and named by Taschenberg *Monocotyle myliobatis* in 1878, is somewhat like the form found in the gills of *Dasybatus pastinacus*, common sting ray, for which I propose the name *Monocotyle dasybatis*, yet as far as can be made out by the description and the rather imperfect portrayal of the anatomy there are fundamental differences which make it necessary to claim for *Monocotyle dasybatis* the right to be called a new species. This latter is nearly twice as large and the mouth, sucker disc and cirrus are different. *Monocotyle ijimae* is also different since it has three testes and a very different cirrus. The head of *M. dasybatis* is 1 mm. in diameter and is composed of one large terminal sucker, trumpet shaped and facing ventrally. Near where it terminates in the pharynx there are three lobulated portions, one on each side, larger than the median one; however, they all concentrate in the pharynx and the whole structure is smooth and unarmed except that along the external margin is a narrow row or rim of muscular cells and minute tubes which may assist in the power of suction or in maintaining its position when fuctionating. The whole head is cordate in shape with the extreme anterior end cut more or less square. The whole structure shows many muscular fibres running through its walls in many directions and these no doubt control the functions and movements of this ample mouth.

The pharynx is large and receives directly the small end of the bell-shaped anterior sucker. It is oval in shape with a median ridge or line running longitudinally from before backward and on each side are eight or nine transverse ridges, giving it the appearance of muscular strength. The postpharyngeal oesophagus is short and divides as usual into two ceca which pass along the sides of the worm posteriorly as far almost as the anterior margin of the sucker disc.

The genital pore is placed near the division of the ceca in the angle formed thereby. It is large and oval and has a patch of spicules on its anterior and posterior edge.

The testis is large, roughly triangular and somewhat lobulated with its base directed anteriorly from near the centre of which the vas deferens arises and proceeds in a winding course toward the gen-

ital pore where it terminates in an armed cirrus which overhangs the genital pore. At about two-thirds of the length of the vas from its origin it widens into a large vesicula seminalis. It then narrows again until near the genital pore where it passes into and through an oblong bulbus ejaculatorius terminating in an armed penis, which organ is comparatively large and thick. It is curved and has a cluster of spicules at its tip.

The ovary is a more or less horseshoe-shaped tubular structure with the concavity directed forwards and is placed immediately in front of or anterior to the testis. In the concavity formed by the curvature there is situated the genital junction which is about at the middle of the worm. The opening of the vagina is seen about opposite the genital pore in the left vitellarium, and the vagina itself plainly showing its circular muscular fibres, runs in a crooked course to terminate in the base of the seminal reservoir which is a large muscular sac. Its tube joins the oviduct prior to the junction of the vitelline duct. The shell gland is distinct and consists of a number of somewhat large masses connected with tubes of varying length to the ootype. The portion of the uterus following the ootype is wide and its walls are composed of long muscular cells giving through its outer coat a spiny appearance.

The uterus is not long but is relatively wide and terminates near the genital pore in a vase-shaped structure, and the mouth is armed with spicules.

The worm as a whole is elongated and appears to be of a very delicate structure and even when alive is more or less transparent, so much so that the principal organs may be seen and traced pretty plainly. Fig. 2a gives a lateral view of the body form.

The sucker disc is a saucer-shaped structure, concavity ventral. It is 1.80 mm. in diameter and is attached to the body on its dorsal surface at about its center with little or no pedicle. It has two fairly large hooks on its anterior surface, one on each side which extend out to and a little beyond its margin almost transversely. There are eight rough ridges and eight loculi shown on its dorsal surface, two of the latter are much larger than the others, the ridges being chitinous and irregularly striated transversely. The two hooks are each laid in close connection with one of these ridges so as to give them firmness of attachment. The dorsal surface of the disc betweeen the ridges is thickly covered with small irregularly-shaped plaques, somewhat like squamous epithelium. The disc as a whole has a definite margin, but extending beyond this is a delicate narrow rim of mucous membrane, and in or on this membrane are delicate, cone-shaped bodies or tubes which are placed regularly side by side around the whole structure, but not extending quite to the extreme outer margin. These cone-shaped portions are crossed by numerous delicate transverse markings or striae. Again immediately within the bases of these cones is

another rim of what appears to be a narrow band of muscular tissue about half the width of the band of cones and the fibres of which are directed regularly in parallel lines toward the bases of the cones, and they doubtless control the movement of the cones as a whole or in part. Other than the transverse ridges on their surface the cones appear to contain a fine granular matter. A somewhat similar structure surrounds the mouth or anterior trumpet-shaped end, although the divisions are not so pointed, and they rather give the impression that they function as sensory areas, although each is composed of three or four deeply stained cells which have fine ducts or lines running towards the centre of the structure or throat, possibly for the secretion of mucus.

The vitellarium is profuse and very beautifully arranged in golden colored masses. A bluntly triangular, shrunken, yellow egg is seen, and in connection with one angle a chitinous filament attached to it does not seem long. The egg is situated at the genital opening.

For the purposes of classification it may be said to be elongate, head cordate in shape with a large trumpet-shaped unarmed mouth, with narrow margin or rim of cells, no large sensory areas, sucker disc with eight ridges, two large hooks, eight loculi, and ornamented with a rim or margin of muscular tubules. Found on the gills of *Dasybatus pastinacus*.

Measurements of *Monocotyle dasybatis*.

Length	7 to 8.00 mm.
Width	1.70 mm.
Width of head	1 to 1.20 mm.
Diameter of sucker disc	1.65 to 1.80 mm.
Length of testis	.80 mm.
Width of testis	.20 mm.
Egg length	.06 mm.
Egg width	.05 mm.

Monocotyle dasybatis minimus, nov. sp.

(Fig. 3)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Mont.).
Family *Monocotylidae* (Tasch.).

On July 17th, 1915, there were found twenty or twenty-five specimens of a small worm on the gills of a large sting ray which it is thought has not hitherto been described. It measures 1.60 mm. in length by 0.37 mm. in width and in situ is invisible to the naked eye.

It is elongated and somewhat triangular in shape, being much wider posteriorly than at the mouth. It is very convex dorsally and correspondingly concave ventrally, although in life quite capable of extension so that the above mentioned shape does not hold except in quietude. It is provided posteriorly with a relatively large sucker disc which is circular and quite as wide in diameter as the widest part of the body. The disc is divided into eight irregularly rounded loculi by eight rough ridges. The posterior end of the body of the worm is implanted on the dorsal surface of the disc at its middle without any pedicle. There are two fairly large much curved hooks on the anterior or ventral surface of the disc. It is also supplied with a number of minute hooks, eight on its margin, which appears to be reinforced by a narrow muscular rim to which the small hooks are attached. The whole skeletal part of the disc appears to be rough and of a chitinous character. The two larger hooks are attached each to one of the strong rough ridges which gives greater power of support. The whole disc is connected to the body of the worm by rather long muscular bands through which it is completely under control, holding the body firmly in place during the performance of its life functions. Its dorsal surface is quite ornamental.

The mouth is subterminal, circular and surrounded by thickened lips, one portion too appears to be more protrusible than the rest. It is very like that in a distom and no doubt acts as a sucker when required to do so. The prepharyngeal oesophagus is short and muscular, terminating in a relatively large and muscular pharynx which is distinctly marked by transverse ridges on each side of its surface. The postpharyngeal oesophagus is also short and, as usual, divides into two ceca which run backwards along the sides almost to the sucker disc. Anteriorly in the angle formed by the division is the genital pore which is generally overhung by the male genital apparatus, the ejaculatory duct, cirrus, etc. Nearby is generally seen a collapsed egg which then appears to be triangular (Fig. 3), and is provided with a filament attached to the posterior pole. It is yellow and chitinous and always single.

The single testis is situated just behind the ovary in the centre of the body—a relatively large vas deferens runs in an irregular course anteriorly, winding around the bulbus ejaculatorius, finally entering the posterior end of that organ and passing through it, terminates in a long curved chitinous cirrus which expands into a trumpet-shaped point at its end. The ovary is a tubular shaped organ placed far back across the body and just in front of the testis. Its concavity looks forward or anteriorly and in the space one of the most conspicuous organs is seen, a large seminal reservoir, very muscular, and supplied from a vagina whose inlet is situated on a small papilla on the right side nearly opposite the mouth of the uterus and terminates in the seminal reservoir with which it is connected at its base. The vitellaria are very

plentiful and extend to the extreme end of the body on both sides from opposite the pharynx anteriorly.

The genital junction is situated at about the middle of the worm and is quite distinct; the vitelline ducts arising at about the junction of the anterior third with the posterior two-thirds of the body proceed backward and internally until they meet the oviduct directly in front of the seminal reservoir which sends its tube to the oviduct at this point and almost directly after this the ootype appears surrounded by the shell gland. The uterus is rather short and terminates in the genital pore on its right side when the worm is viewed dorsally.

Measurements of *Monocotyle dasybatis minimus.*

Length	1.60 mm.
Width	.40 mm.
Diameter of disc	.40 mm.
Egg	0.06 mm. + 0.05

For classification it may be said to be elongate, wide at the base or disc and narrow at the apex or mouth. Mouth surrounded by a thick roll or lip, one side of which is prolonged to a point. Disc composed of eight loculi and eight ridges—two large and eight small hooks, and the whole dorsal surface of disc ornamented with roughened striae of chitin.

Merizocotyle dasybatis, nov. sp.

(Figs. 4 and 4A)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Mont.).
Family *Monocotylidae* (Tasch.).
Genus *Merizocotyle* (Cerf.).

On August 1, 1914, while examining a very large sting ray *Dasybatis pastinacus*, at the United States Fish Commission's Laboratory at Wood's Hole, Mass., I found in the nasal mucus glands a number of odd forms. They were embedded in the profuse thick mucus which is always present in or near these glands. Many were found deep in between the laminae of the gland and in some cases were rather difficult to detach.

It has been customary for former writers to state that these trematodes as well as many of the acanthocotylidae have their habitat on the skin and gills of their hosts, but I am sure from the numbers of them that I have seen in the nasal glands of rays, skates, etc., that their natural and permanent habitat is there and that the other locations are merely accidental.

This form has been placed in the family Monocotylidae, Tasch., and the genus *Merizocotyle* (Cerf.). Of the few who have observed this worm Cerfontaine has written the best description which is available to me. His description of *M. diaphanum* and *M. minus* seems quite complete and enables me to know that the species herein described is not either of these, as will be seen from the following description:

The worm for which I propose the name *M. dasybatis* is 2.40 mm. in length by .60 mm. in width. It possesses a large almost circular cancavo convex sucker disc as wide or nearly as wide as any part of the body. The body is elongated, concave from end to end and from side to side ventrally and convex dorsally, and is somewhat constricted anterior to the sucker and at the neck. The head is triangular in shape and divided by a deep notch which extends posteriorly from the tip nearly to the pharynx. On each side of the notch on the ventral surface are three quite large tactile areas or papillae and running backwards from each of these is seen a wide duct disappearing under the vitellaria.

The mouth is a peculiar protrusible tube at the anterior opening of the pharynx and forming when quiescent merely a thickened rim of circular folds. It conveys the impression of being capable of considerable extension and of suctorial power. The pharynx is goblet-shaped and large; it is surrounded by a large number of unicellular glands in masses distributed posteriorly almost as far as the genital pore. On the dorsal surface over the pharynx are four ocular spots.

The intestines seem wide and are given off behind the pharynx without any great prolongation of oesophagus and at their bifurcation the genital opening appears surmounted by the chitinous cirrus.

The male genital apparatus.—The testis is single, pear-shaped and large, but not so disproportionately large as in *M. diaphanus* and minus. It is situated at the junction of the posterior third with the anterior two-thirds of the body encroaching upon each division. The vas deferens passes anteriorly along the left side until nearly opposite the middle of the short uterus and then expands into a large vesicula seminalis; from this it passes forward to the base of and through the bulbus ejaculatorius a round muscular organ at the base of the cirrus which surrounds the vas deferens in this portion of its course.

The cirrus is a curved hollow chitinous tube slightly turned up at the tip. In the bulbus ejaculatorius beside the vas deferens passing through it may be seen at its base and on each side a small gland which sends a tube forward toward the base of the penis.

The female genitalia consists of a twisted horn or flask-shaped organ (the ovary) with a long neck, which, after coiling upon itself once, proceeds forward to the genital junction about the middle of the worm, after which the oviduct widens into a wide ootype, and this in turn merges into the uterus.

The ootype is an unusually wide, thick portion of the genital canal and is lined with a layer of large, elongated, deeply-staining cells. In this instance it contains an almost completely developed egg which seems relatively large; it is yellow, oval and with a moderately long filament at each pole.

The uterus is short and muscular and terminates in a thick-lipped flaring mouth at the edge of the genital opening, situated centrally near the cirrus and bulbus ejaculatorius.

There are two vaginae opening ventrally, one on each side near the margin of the body. They join in the middle of the body in the form of a hammock, the central portion being the seminal reservoir. Towards the outlet of each vagina its walls contain circular muscular fibres which act as a constrictor. This whole structure is slung across the body at the posterior end of the ootype and very near the genital junction.

The shell glands are large and with long necks, but not excessively numerous.

The vitellaria are plentiful and extend from the base of the pharynx almost to the disc posteriorly. It covers the intestinal ceca from view until near the disc.

The disc, or sucker, is large and powerful and is composed of several suckers and hooks.

It is nearly circular and concavo convex structure, the ventral surface presenting five central loculi, one small deep one in the centre and four larger ones surrounding it. On the margin there are twelve suckers beside the large triangular space at the extreme posterior, formed by the divergence of the very large pair of hooks situated here. This space appears to have four small suckers near its margin, and there are also here four small hooks, one for each sucker. There are two long straight spicules here which run to the two middle small hooks. Each sucker around the rest of the margin of the disc is supplied with a small hook.

Measurements of *Merizocotyle dasybatis*.

Length	2.40 mm.
Width	.60 mm.
Disc, diameter	.30 mm.
Hooks on disc, length	.17 mm.
Testis	.11 x .08 mm.
Ovary to beginning of genital connection	.35 mm.
Egg	.04 x .03 mm.
Cirrus, length	.05 mm.
Pharynx	.07 x .05 mm.

Trionchus dasybatis, nov. gen. et nov. sp.

(Fig. 5)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Mont.).
Genera *Monocotyle* (Tasch.).

This worm was found on the gills of a very large sting ray *Dasybatus pastinacus*, on August 1, 1914, at Wood's Hole, Mass., and was studied at the Laboratory of the United States Fish Commission there.

Only two specimens were found, as the gills were very large and thickly covered with mucus, so that the worms on account of their small size (1 mm. x .60 mm.) were easily overlooked. Besides, their attachment is exceedingly firm, so that few were brushed off. When first seen they were very active, alternately shutting up their discs toward their heads and again relaxing them. When closed they reminded one of a trilobite. The body is plump, very concave in all directions ventrally and very convex dorsally.

For the purpose of classification the following characters may be given: The mouth large and subterminal, much like an ordinary sucker; genital pore central; cirrus chitinous; single testicle posterior to ovary; a relatively large sucker disc with one large loculus in the centre and three small marginal ones. There are also on the disc three hooks, one large one terminating in two points and also two smaller ones.

The mouth is large and circular occupying almost the whole width of the anterior end. The lips are thick and could act in the capacity of a sucker when necessary.

The pharynx is very near the mouth, a muscular barrel-shaped structure divided into two halves on the surface by a central antero posterior line or groove, and each half shows transverse lines which apparently divide it into seven bands of muscular tissue.

The vitellaria reach on each side to about the middle of the pharynx and extends posteriorly along each side of the disc for a short distance.

Immediately posterior to the pharynx and surrounding to a great extent the short post pharyngeal oesophagus and its bifurcation into the intestinal ceca, is a mass of unicellular glands which supply these structures with mucus. The intestinal ceca extend to near the junction of the middle with the posterior third of the body.

The male genitalia are quite distinct. The testis is situated in the posterior portion of the body just behind the ovary; it is fairly large and somewhat pyriform in shape and from the small anterior end there arises the vas deferens; this empties into the flask-shaped vesicula seminalis. The vas deferens continues as a narrow tube for some distance, when it widens again into a fusiform enlargement the ductus ejaculatorius and from the anterior

FIG. 5. *Brontosaurus excelsus*. Type. Ventral view of skull.

FIG. 6. *Brontosaurus excelsus*. Type. Dorsal view of skull.

FIG. 7. *Brontosaurus excelsus*. Type. Occipital view of skull.

end of this it extends to the base of and through the bulbus ejaculatorius to terminate in the cirrus. The bulbus ejaculatorius is a more or less round muscular ball or sac, the name of which indicates its function.

The cirrus is a slightly curved, relatively long, yellow chitinous tube, the base of which is applied to the bulbus ejaculatorius by means of three spreading arms or branches of chitinous material covered in with muscular tissue, etc. The tip end terminates in two or three spiny processes.

The ovary is large and somewhat semilunar in shape with the concavity looking anteriorly and one end is prolonged into an oviduct. Only the mouth and terminal portion of the uterus is apparent in these crushed specimens. The mouth is funnel shaped. The widened mouth in this case contains a fully formed egg, and is situated near the margin of a genital atrium placed well within the angle formed by the bifurcation of the intestinal ceca and is therefore central.

The egg is irregularly oval, yellow, and has a filament attached to its anterior pole. This filament is rather less in length than the egg itself, which measures .045 mm.

The sucker disc on its ventral surface is concave and oval in contour. It is seen to be divided into one large deep sucker occupying most of the disc and three small marginal ones. In the centre of the margin the largest of these smaller suckers supports through its centre the largest of the three hooks, and extending out from it is a tongue-like process of skin which also helps to support the central large hook. These are the only hooks on the disc. The large one is three times as large as the lateral ones, which are more curved than the large one. The large hook is .07 mm. long and terminates in two sharp hooklets. The marginal skin is extended outward opposite each of the other loculi, but not to the same extent as in the case of the central one.

In life the sucker disc is frequently clasped to the front end of the body and by this movement the progression of the worm is in some way facilitated; it may be much as a crab or lobster flaps its tail.

In the narrow portion between the body and the disc many muscular fibres are seen extending up into the body. Excretory canals can also be seen, but no outlet could be made out.

Measurements of *Trionchus dasybatis*.

Length	1.00 mm.
Width	.60 mm.
Sucker disc, width	.25 mm.
Sucker disc, length	.14 mm.

Long hook on disc, length	.070 mm.
Short hooks on disc, length	.025 mm.
Cirrus, length	.040 mm.
Egg, length	.045 mm.
Egg, width	.035 mm.

Monocotyle selachii, nov. sp.

(Figs. 6 and 6A)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Grout.).
Family *Monocotylidae* (Tasch.).

On July 19, 1915, at the Laboratory of the United States Fish Commission, Wood's Hole, while examining the nasal glands of a large *Cestracion zygaena* there was found within them a *Monocotyle* which is regarded as a new species.

It is elongated in form and almost of equal width throughout including the head and disc. The head is of an irregular square shape with four prominent enlargements, two on each side of a concavity at the anterior margin which does not, however, denote the location of the mouth. The enlargements are evidently sensory areas and probably are capable of some extension when the worm is feeling its way about. However, there does not appear to be any suctorial arrangement by which the worm is enabled to maintain its position, if we leave out the mouth at the anterior portion of the pharynx. Almost in the centre of the portion called the head is seen a more or less square shaped structure which represents the pharynx with the mouth in front. The mouth and perhaps part of the pharynx is protrusible as needed. This is shown by the thickened and movable lips. The mass also includes the origin of the ceca which start from the posterior lower part of the pharynx and pass almost at once behind the vitellaria, which hide their course backwards. A very short distance further posteriorly a coil of the vas deferens appears before it makes its way to enter the rounded end of the bulbus ejaculatorius which in this instance is directed toward the pharynx and is oblong and large relatively. From its other end projects the cirrus which is rather short and wrinkled and somewhat triangular, point blunt and quite rounded. It is covered with an outer shell, and is probably protrusible. The whole structure in this instance lies anterior to and over the genital pore.

The vas deferens passes anteriorly along the right side, and shows it to be full of spermatozoa.

The testis is either enclosed in a sac and lobulated, or it consists of several small masses or testes. The posterior end of the worm is so filled with vitellaria that a clear view of this organ is impossible—an examination of further and better material may clear up this point.

The ovary is situated near the middle of the body, and is somewhat S-shaped. One end, however, is thick and massive, while the anterior portion is prolonged into a long narrow coiling neck, which in one instance at least, reached nearly to the genital pore. This, however, seemed to be exceptional, since the oviduct arises generally from the anterior portion of the organ and almost at once receives the vitelline ducts. No seminal reservoir could be made out in the material examined, nor could a vagina be traced, although an opening could be seen on the right side of the genital pore, very near the vitellarium, which seemed to be the mouth of a vagina. The vitellaria are plentiful and extend from the pharynx to the caudal disc, being unusually profuse posteriorly.

The sucker disc is a large structure of unusual formation. It is armed with two short strong and much curved hooks on the anterior surface, and in the worms from the *Carcharias obscurus* there are also a number of very small hooks on the extreme margin, but so far no small hooks could be seen on the edge of the disc in the case of those found on *Cestracion zygaena*, although otherwise the worms seemed alike. The ventral surface of the disc has scattered over it a great number of small circular loculi or suckers of different sizes, and which probably have different or separate innervation which may enable the worm to have greater power, or to release its hold partially at will; be that as it may, the fact is that this disc has only two large hooks besides, along the margin, several small ones placed at regular intervals. There are no ridges, and the worm must depend on its large sucker for support in its place of attachment, assisted by the powerful pharynx and mouth, where there appears much muscular tissue. It is probable though that as the nasal gland is its local habitat enclosed from outside influences and fixed in profuse sticky mucus, it does not need any greater means of adhesion. Some writers have described the findings of worms of this class on the skin and in the mouth of their host, but it must be that they are only accidentally in these situations, their proper home is in the much protected nasal gland.

For the purpose of recognition and classification it may be said to be oblong, even in width throughout, sucker disc with two hooks, several very small ones on the margin and no ridges, the ventral surface covered with numbers of small suckers of different sizes, body very delicate and fragile. Found in the nasal gland of *Cestracion zygaena* and other sharks and no where else so far; genital pore central. The skin is unarmed and the body is sparsely covered throughout with small cells, muscular tissue thinly distributed except at the junction of the disc and the body, water vascular system quite evident, on each side of the pharynx are to be seen small ducts which develop into quite large canals which show as an open line on each side in the dense vitellaria. They terminate near the disc, but no definite outlet is seen. Since the same worm is also found in the

nasal passages of *Carcharias obscurus* as well as *Cestracion zygaena*, and, as I have no doubt it may be also found in similar locations in other sharks, I would therefore propose the name *Monocotyle selachii*.

Measurements of *Monocotyle selachii*.

Length	5.40 mm.
Width	1.00 mm.
Width of head	1.20 mm.
Width of disc	1.00 mm.
Length of disc hooks	0.03 mm.

Diplectanum plurovitellum, nov. sp.

(Fig. 7)

Sub-family *Gyrodactylidae* v. Ben. et Hesse
Genus *Tetraonchus* v. Ben. et Hesse
Sub-genus *Diplectanum* v. Ben. et Hesse

A new form of *Diplectanum* was found on the gills of *Teuthis hepatus* on November 27, 1915, which seems to have escaped observation hitherto. It has also since been found on the gills of *Anisotremus virginicus*.

Like all the other members of the family it is small, being only 1.60 mm. in length by .20 mm. in width. Its head is more or less conical in shape, much more so than any of the other known members of the family, but the most striking feature of its anatomy is the peculiar arrangement of the vitellaria which is distinctively blocked, cut into two rows, one on each side of irregular more or less triangular blocks distinctly separated from one another, except that along the internal margin of each row, where they are connected by an antero-posterior column of vitellarium. There are generally sixteen or seventeen of these portions on each side, and they extend from nearly as far forward as the pharynx and to near the disc posteriorly. The mouth is not definite but the pharynx is large and prominent and probably serves as the mouth. On each side of this extending from about its middle on each side is a row of tactile areas which seem to serve as sensory areas. They extend nearly to the extreme anterior end of the worm. There are four ocular spots, two just within the edge of the pharynx and two more anteriorly. The bifurcation of the oesophagus takes place back of the pharynx, and is not very distinct, although it can be made out to a certain extent in the usual situation and a short distance posterior to this division may be seen the genital pore, where the uterus terminates.

The uterus is short and evidently only contains one egg at a time for in each specimen under observation the uterus contains an ovoid, yellow egg which is relatively large; it is without the

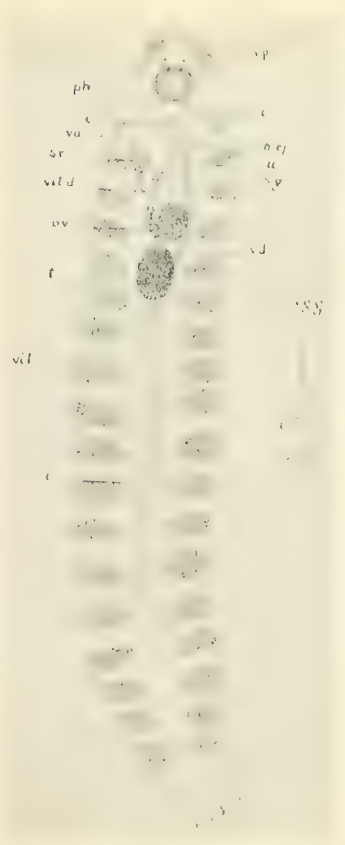

FIG. 7. *DIPLECTANUM PEUROTITTUM*. c. Cirrus.

FIG. 8. *ICHTHIODISCUS REMORE*.

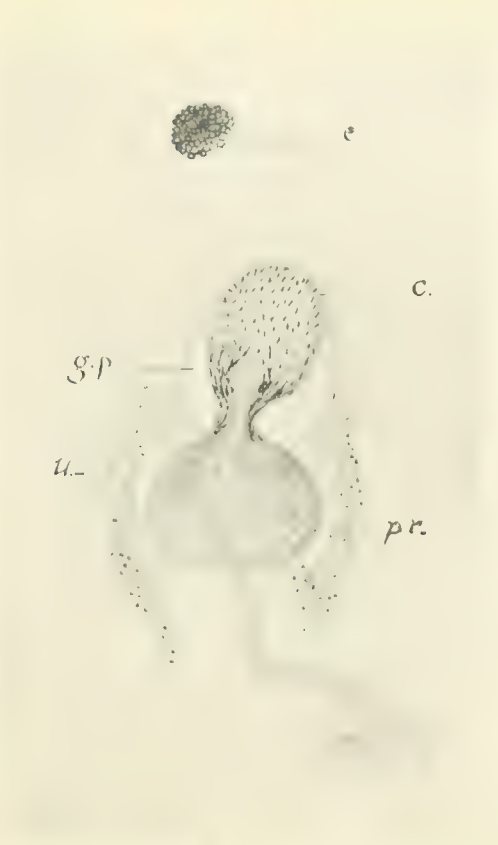

FIG. 8 A. CIRRUS AND PROSTATE OF *I. REMORE*. e. Ejaculatory duct.

usual filament. Over the genital pore the cirrus presents itself and is peculiar in form being in this quite as diagnostic as those of the other species. It is long and curved and has a bulbus ejaculatorius at its posterior end which is more or less pear-shaped, the larger end being posterior. The ovary is roundish or rather ovoid and is centrally situated at the junction of the anterior quarter with the posterior three-quarters of the worm, and immediately behind it is the large single roundish testis. The genital junction is directly in front of the ovary. The vas deferens passes on the left side of the ovary towards the posterior end of the bulbus ejaculatorius.

The intestines or ceca seem to join into a single tube posterior to the testis which passes down the middle to nearly the posterior end of the worm.

The disc is directly terminal after the body has narrowed somewhat. It is armed with two large and strong hooks which point laterally and at least also with eight spicules or small hooks on the posterior edge of the end of the disc. The skin is smooth and unarmed.

On the gills of the same host were a number of *Diplectanum teuthis.*

Measurements of *Diplectanum plurovittellum.*

Length	1.60 mm.
Width	0.20 mm.
Egg	.05+.02 mm.

Acanthodiscus remorae, nov. sp.

(Figs. 8 and 8A)

Order *Trematoda* (Rud.).
Sub-order *Heterocotylea* (Mont.).
Family *Gyrodactylidae* v. Ben. et Hesse.

On May 16, 1915, while examining some material from New York Aquarium I found on the gills of an *Echeneis naucrates* a small worm as well as a lot of specimens of *Dionchus* (Goto). Again on May 19, more attention was paid to the gills of a larger fish, which in order to secure more specimens of the new worm, the gills were rubbed under water between the fingers and thumb, and the result proved to be the dislodgement of a number of the worms sought although a good many had left their discs adhering to the gills. Several, however, were perfect and answered the following description: It is small, not more than 1.80 mm. long and .30. mm. wide. It is elongated and presents as its chief characteristic a relatively large posterior clinging disc on which are two large much curved hooks. These seem to be attached to a loose marginal fold around the edge of the disc. The disc is concave and presents on its sur

face a series of irregular depressions, but no ridges may be seen. It merely looks like one large sucker with near its center two large hooks, one on each side, much curved and with the points looking toward the center of the disc. The skin is thick and unarmed, although it is serrated. The head of the worm is in shape that of an obtuse triangle with the small mouth terminal; a short distance from the mouth is seen the pharynx and between the two are four ocular spots. Around the ocular spots and also enclosing part of the pharynx is a circle of deeply stained mucus cells three or four deep. The head is somewhat marked off from the body by a notch on each side conveying the impression of the presence of a neck and it is surrounded by a margin of elongated mucus unicellular glands so closely set together, and staining more deeply than the neighboring tissue that it gives the appearance of a narrow band extending as far as the neck a little back from the edge of the worm. Inside of this band of cells is a narrow, clear space, where the water vascular ducts are plainly seen also surrounding the head.

The pharynx is roundish, and it is surrounded by a circle of mucus cells which apparently supply it with mucus. On the left side of the neck the genital atrium is placed, a short distance from the edge. It is sparingly armed with a cluster of spicules. This opening is almost directly opposite the pharynx, and shows the cirrus and uterus there. There are two testes, one before the other in the center of the body, and either one is twice as long as the ovary, which is situated in front of them. They are oblong and rounded and give forth a relatively very large vas deferens which is much coiled and makes its way anteriorly, first emptying into a large vesicula seminalis then into a large cirrus sac. The cirrus itself is apparently armed at the end, and is more or less globular at this point with a large prostate behind. The ovary is small and round and situated about the middle of the worm. The oviduct is fairly long, and is met by a duct from the seminal reservoir situated on the left side and in front of the ovary; but if there is a vagina it must be back from the margin of the worm among the vitellaria, for it cannot be made out distinctly. The ootype is surrounded by a voluminous shell gland, and the uterus near its termination is provided with a metraterm well developed. The intestines are not easily followed in their course under the profuse vitellaria which extends from the neck on each side to near the disc posteriorly. No fully developed eggs were seen.

Measurements of *Acanthodiscus remorae.*

Length	1.80 mm.
Width	.30 mm.
Diameter of disc	.40 mm.
Width of neck at eyes	.20 mm.
Diameter of ovary	.10 mm.
Diameter of testis	.20 mm.

Fig. 9

Fig. 9-A

Fig. 9-B

FIG. 9 — *ICHTHIOCOTYLE RUBE*. FIG. 9-A. LATERAL VIEW. FIG. 9-B. NASAL GLAND.

Acanthocotyle raiae, nov. sp.

(Figs. 9 and 9A and B)

Sub-order *Heterocotylea* (Mont.).
Family *Gyrodactylidae* v. Ben. et Hesse.

On July 4, 1914, while examining a large summer skate, *Raja erinacea* at the Laboratory of the United States Fish Commission at Wood's Hole, there were found in the nasal mucus sacs or glands a number of small forms for which I propose the above name.

The nasal gland of fishes seems to have received scant attention and the fact that it is a very favorite home for certain species of Heterocotyleans and even some crustaceans as Copepods, Ergasillae, etc., seems to have been entirely overlooked. The wide open nostrils of course are always noticed, but behind these are the large mucus glands whose function seems to be to secrete large quantities of mucus, perhaps to assist the numerous skin glands in the anterior portions of the fish to lubricate the front and thus facilitate speed, lessen friction, etc. In *Raia erinacea* the gland is a little over two inches long by a little over an inch wide and is more or less cylindrical. It is open at one side and closed by a broad, band-like structure on the opposite side which runs from end to end. Each side is composed of many thin lamellae placed closely side by side across the length of the structure which allows it to be opened like a book although the leaves run in an opposite direction. It is generally filled with thick mucus and in this the worms live. (Fig. 9b.)

To show that the location where found is the natural habitat, I may say that in this instance as many as twenty-five worms were found in the two glands. They were also found in the same location in other skates of this species, as well as in *Raja ocellata*. It has been customary with parasitologists to credit this family of small worms as skin parasites, and indeed they may sometimes be found on the skin, but I am confident that their position there is merely accidental, for as may be seen by the description of its anatomy it could not derive any nourishment on the outer skin of these large and rough fishes, nor could it even retain its hold there for any prolonged time; while embedded always in the plentiful mucus of the nasal glands, they not only find plenty of nourishment, but are protected from outside exposure.

The present member of the group is only 1.60 mm. in length by 0.60 mm. in width. It is elongated in form, delicate in structure and of nearly equal width throughout. It is concave ventrally and very convex dorsally. (Fig. 9a.) Its most prominent feature is a large roundish sucker disc at its posterior extremity. Its head or anterior end is but little curved from side to side, and in the centre is a shallow notch, on each side of which are three sensory papillae. A short distance posterior to the notch in the anterior

border is a prominent pharynx, the anterior end of which has thickened lips which constitute the mouth of the worm.

The oesophagus is short and divides into two wide intestinal ceca which extend well back towards the disc. The disc is round and concavo-convex having on its ventral surface fourteen suckers of medium size around the margin and five larger ones in the center. These are separated by prominent rough ridges, indeed every loculus is surrounded by a very rough margin. At or near the outer edge of the space between each pair of marginal suckers is a very small hook. The disc is almost as wide as any part of the body being 0.39 mm. in diameter.

The male genitalia consist of two relatively large oblong testes which lie side by side in the middle of the body and immediately behind the ovary. The vasa deferentia unite and run coiling forward to form a prolonged widened portion or sac,—the vesicula seminalis. This sac shortly narrows to a small tube which again widens into a fusiform structure, the ductus ejaculatorius, which in turn narrows and enters the base of the bulbus ejaculatorius; passing through this organ it terminates in the cirrus a long curved organ which flares somewhat at its extremity. It is chitinous in structure and somewhat yellow in color, it overhangs the genital pore and is apparently always directed backwards.

The female genitalia consists of an irregularly curved flask-shaped body, the ovary with a long neck. Its base rests between the anterior ends of the testes and the neck extends forward toward the genital pore coiling somewhat on its way. The genital pore is situated centrally in the angle formed by the beginning of the ceca. The neck of the ovary ends in the oviduct which shortly receives the vitelline ducts projecting from the vitellaria on the sides. The seminal reservoir lies across the worm a short distance posterior to the uterus. It is in the shape of a hammock having a vagina connected with it on each side. It communicates here with the oviduct which passes on to pass through a large shell gland and then merges into a peculiarly shaped uterus which is somewhat fusiform or pear-shaped, the widest portion being anterior and displaying there its mouth with very thick lips. The uterus seems to be constructed of large muscular cells which stain deeply. It seems only constructed to hold one egg at a time and in one specimen an egg was seen to occupy the mouth. This was oval, somewhat collapsed, yellow and with a short chitinous filament attached to one pole. The egg measures 0.02 mm. (Fig. 9.) The vitellaria are very plentiful, extending along to the sides from opposite the pharynx to near the disc.

For the purpose of classification the worm may be said to be elongated, to three times its width, although this varies much in life, disc without large hooks, but with fourteen marginal loculi,

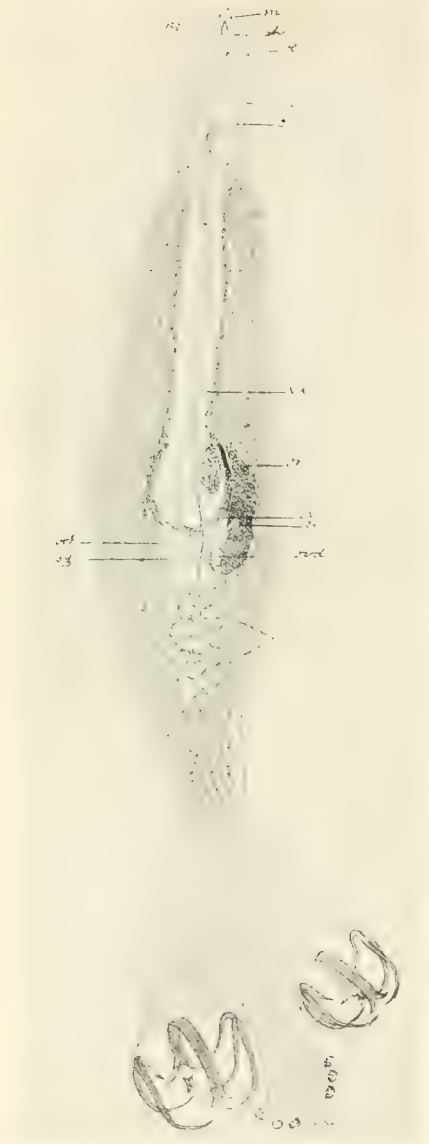

Fig. 10

FIG. 10.—*INTHOCOTYLE MERLUCII AMERICANUS*

Fig. 10-A

FIG. 10-A. GENITAL SCHEME OF *A. MERLUCII AMERICANUS*.

b—One of the large suckers or claspers. d—Hooks at tip of disc.

five internal ones, and several small hooks. Occurs in the nasal glands of several species of skates.

Measurements of *Acanthocotyle raiae.*

Length	1.60 mm.
Width	.50 mm.
Diameter of disc	.30 mm.
Egg	.02 mm.

Anthocotyle merlucii americanus, nov. sp.

(Figs. 10 and 10A)

Family *Octocotylidae* v. Beneden et Hesse.

At Wood's Hole, Mass., at the Laboratory of the United States Fish Commission on August 1, 1913, I found on the gills of *Merluccius bilinearis* a worm which in general appearance resembles the *Anthocotyle merlus,* or *merlucii,* first described by Van Beneden and shown by a plate given in his paper and also by Dr. M. Braun. This plate, however, shows no anatomy of the worm except in the most general outline. On studying the present form more closely I found that while of the same genus it was not by any means the same worm or the same species. In the first place this is larger and the first two large suckers which are such a prominent feature in the form are not at all like those of *A. merlucii* of the European hake either in their shape or structure. Again, while *A. merlucii* has only the eight suckers usual with the Octocotylinae the new worm has beside these a small square shaped extension of skin between the posterior pair of suckers on which are placed four small hooks and which form the extreme posterior limit of the worm. These differences have induced me to ask a place for the American form as a new species.

The worm is found in sparse numbers only as far as I know on the gills of a common well known fish on the New England coast,—the New England hake, or whiting, *Merlucius bilinearis.* This fish is common in American waters from the Bahamas northward to the Straits of Belle Isle.

Anthocotyle is a worm of a very delicate, thin structure and yet with very powerful means of holding to its place of attachment, and this is probably the reason for its described rarity. Van Beneden claims only to have seen two specimens. However, if care is taken they may be found. It is extremely filmy except in the neighborhood of its large suckers, where there is considerable muscular tissue, as well as the large chitinous framework of the suckers.

It belongs to the family Octocotylidae and the peculiarity of its armature is that while it has eight suckers, the two anterior ones are out of all proportion with the others of the set, the anterior ones being very large while the other six are very small. The anterior ones also are not of the same size, the right one being much larger than the other, and this is constantly so in all the specimens. (Fig. 10.)

The worm is about 9 mm. in length by 1.20 mm. in width at its widest point; one specimen, however, measured when mounted 12 mm. by 2 mm. The large suckers measure in diameter 1.20 mm., while the smaller ones measure .053. The head is very small, being surmounted on each side by a rather large sensory papilla. The mouth is large and semilunar in shape, the concavity being toward the anterior extremity. Somewhat posterior to the lower lip on each side is a small sucker, and still more posterior and between these is the pyriform pharynx. The oesophagus bifurcates almost immediately into ceca and in the angle thus formed is as usual the genital opening, or uterine atrium; protruding from this is a very prominent cirrus at the end of the vas deferens. The end of the cirrus is enlarged and also armed with rather long chitinous spines or spicules arranged in somewhat irregular rows. The vas deferens is large and more or less occupies the central space between the profuse vitellaria, which occupies the greater part of the body on each side. The testes not very numerous, are small and are almost entirely covered under the vitellaria. However, in one large specimen twenty could be counted.

The female genital apparatus consists of the usual organs; the ovary is club-shaped and situated at about the middle of the body, the oviduct being a continuation of the smaller end which is at first directed posteriorly, but after receiving the duct from the seminal reservoir and the vitelline ducts it proceeds across the body from left to right, to pass into the ootype. The shell gland is small and compact and very distinct. The ootype is fairly large, as is also the uterus. In one of the specimens being described a fully formed egg is seen. It is long and narrow and provided with a filament at each end which are several times the length of the egg as shown in figure 2. At the point where the worm suddenly enlarges at the neck, forming a sort of shoulder, is the opening on each side of a vagina, both of which can be traced posteriorly as far as the beginning of the vitelline ducts.

One of the main points of difference between this form and that of *A. merlucii* is first that the chitinous structure in the suckers is very different, as may be seen by the plate, and second that between the last pair of small suckers a prolongation of skin, square across the terminal and is armed with four different hooks. (Fig. 10a-b.) These are entirely absent in *A. merlucii*. The size of *A. merlucii*,

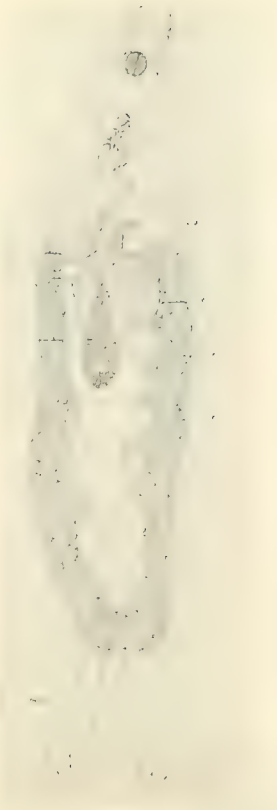

Fig. 11

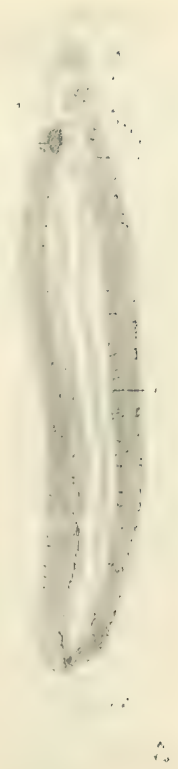

Fig. 12

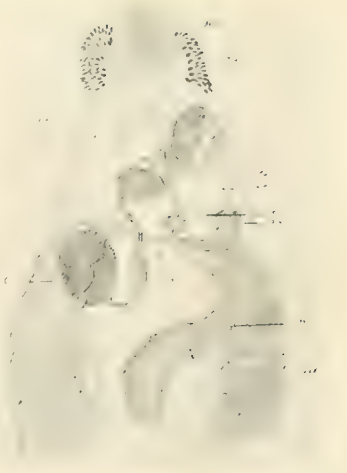

Fig. 12 A

FIG. 11. *AMPHIBOLITE TORPIDUS*. FIG. 12. *AMPHIBOLITE EUROPINEUS*. FIG. 12 A. GENERAL SCHEME OF *AMPHIBOLINEUS*.

4 mm., as given by Van Beneden is not more than half the length of the American form, which in some cases measured 12 mm.

Measurements of *Anthocotyle merlucii americanus.*

Length	9 mm. to 12 mm.
Width	1.20 mm. to 2 mm.
Diameter of large suckers	1.20 mm.
Diameter of smaller suckers	.50 mm.
Egg	.05 mm.

Amphibdella torpedinis.

(Fig. 11)

Family *Gyrodactylidae* v. Ben. et Hesse.
Genus *Amphibdella* (Chatin).

In 1874 Chatin discovered and described this worm with the above family history. He found it on the gills of *Tetranarce occidentalis* and named it *Amphibdella torpedinis.* I am only writing a short description of it in order to compare it with another species of the same family which I found on the same fish and under the same conditions as *A. torpedinis*, a description of which I am giving in a separate paper.

They are totally different, although found in the same habitat in about equal numbers (see plate). As the description of this worm in any book to which I have access is rather meagre and no plate showing its anatomy is given, I am tempted to try and give a little more perfect description as I found it, also in order that it may be compared with *Amphibdella flavolineata* as given in this paper.

The form is quite graceful and about 4 mm. in length by 1 mm. in width. The anterior end is concave, in the center of which is the mouth. On each side of the head are seen three large sensory papillae, and between the posterior two is a mass of unicellular glands, which in the stained specimen is highly colored. There is also a mass of these on each side of the pharynx. The pharynx is relatively large, and the postpharyngeal oesophagus bifurcates into the intestinal ceca close to the pharynx. The genital opening is placed in the angle formed by the branching ceca and here prominently is seen the large peculiar chitinous cirrus, composed apparently of two tubes ending in three brownish hooks at the distal end of the cirrus; at its base, or at least connected with it is seen the bulbus ejaculatorius, through the center of which the seminal tube passes; immediately behind this is the ductus ejaculatorius, which is quite muscular and is somewhat sausage-shaped, although becoming quite small at each end; this receives the seminal duct from another somewhat semilunar enlargement, which may be called the posterior prostatic reservoir; this also is con-

nected posteriorly with the vesicula seminalis, which is the main reservoir of spermatozoa and it is directly in connection with the testis by means of the vas deferens. There is also a fine tube, brown in color, which extends from the junction of the posterior prostatic reservoir and the ductus ejaculatorious; this is seen to enter the cirrus at about the middle of its length. The testis is situated beside the ovary on the left side of the worm when looking at the ventral surface, it is single and the vas deferens crosses over the dorsal surface of the ovary to join the vesicula seminalis.

The ovary is flask-shaped with a long neck, which is in part the oviduct. The genital connection takes place a little anterior to the ovary and the uterus, which is quite short, ends at the edge of the genital pore in the center of the anterior part of the worm. Its mouth is somewhat funnel-shaped and appears muscular and circular.

The vitellaria are plentiful and extend from opposite the anterior part of the ovary to the disc posteriorly.

No vagina is visible. The excretory system may be distinctly seen terminating in two or three good-sized canals which extend to the sucker disc. One small canal is seen to empty on the side of the body.

The intestines seem to end in one large coecum which passes down the middle of the body cavity and ends at the junction of the middle with the posterior third of the body.

The disc is wide and muscular and connected with the body by a neck almost as wide as the body. The muscular fibres which control it are plainly visible. The disc is more or less circular on its distal face and is armed with four large yellow hooks besides several very small hooks situated at the edge of the disc.

Measurements of *Amphibdella torpedinis*.

Length	4.00 mm.
Width	1.00 mm.
Pharynx, diameter	.09 mm.
Pharynx, from anterior end of worm	.20 mm.
Ovary, length	.35 mm.
Ovary, width	.04 mm.
Testis, length	.20 mm.
Testis, width	.07 mm.
Sucker disc, width	.25 mm.
Sucker disc, depth	.13 mm.
Hooks on disc, length	.10 mm.
Chitinous Cirrus, length	.05 mm.
Chitinous Cirrus, width	.15 mm.

Amphibdella flavolineatus, nov. sp.

(Figs. 12 and 12A)

Family *Gyrodactylidae* v. Ben. et Hesse.
Genus *Amphibdella* (Chatin).

In 1874, Chatin discovered and described a small worm belonging to this family, which he found on the gills of *Tetranarce occidentalis* and which he named *Amphibdella torpedinis*.

On July 6, 1914, at the United State Fish Commission Laboratory at Wood's Hole, Mass., there was found on the gills of a large torpedo-*Tetranarce occidentalis* a great many *A. Torpedinis* and also an almost equal number of another *Amphibdella* which as far as the writer is aware has not hitherto been described. It is quite different from *A. torpedinis* and for purposes of identification the following characters may be given: Two prominent rows of light yellow colored, lobulated vitellaria, one along each side; a large armed sucker disc connected to the body by a much constricted neck; disc divided into two large lobes, and these again subdivided into several smaller lobules. On each of the lobes are two fairly large hooks, but as far as can be seen there are no other smaller hooks. The disc is joined to the body by a narrow pedicel or neck, but strong muscular bands may be seen extending from the hooks and disc well up into the body.

The worm is from 4.50 to 6 mm. in length by 1 mm. in width. The genital organs are situated chiefly in the anterior quarter of the body. The head is small and surmounted on each side of the mouth by three sensory papillae or areas. These connect with small tubes which are numerous as far as the pharynx. The pharynx is round, large and surrounded by many vascular unicellular mucus or salivary glands, which by means of long ducts open into the oesophagus and pharynx. They stain deeply and are hence prominently in sight.

The post-pharyngeal oesophagus divides almost immediately into two intestinal coeca which are long and extending down the center of the body throughout most of their course, almost to the base of the sucker disc.

The female genital apparatus occupies the triangular space between the bifurcation of the oesophagus into the intestines and between the anterior ends of the vitellarian tubes. These latter are the most prominent markings of the worm in life, giving it the appearance of having two wide light yellow lobulated stripes throughout nearly its whole length. When magnified these stripes show each to be enclosed in a sac or membrane and to be made up of large rounded lobules of vitellaria. At the anterior end the two columns come together by two slender vitelline or yolk ducts where they enter the oviduct.

The ovary is large, graceful and flask-shaped with a long neck and this terminates in the oviduct which is short and joined by the ducts from the seminal reservoir and vitellaria. The shell gland is also to be seen very distinctly near this point.

The uterus is quite short and ends near the edge of the genital atrium in the centre of the body and well into the angle at the bifurcation of the intestinal coeca. Its end or mouth is at the end of a triangular or funnel-shaped widening of the tube. In the specimen described herein an egg is observed which is oval, yellow, and in this case has a wrinkled chitinous shell. It measures .03 in length and apparently only one egg is developed at a time, for in this case no other can be seen to be in process of formation. No vagina can be seen.

The male genital apparatus also occupies the anterior portion of the worm and is a rather complex structure.

The testis, a large single one, is situated dorsally of the anterior end of the right vitellarium. The vas deferens is consequently short and leaving the testis it widens somewhat, then enters the base of the anterior prostatic reservoir or vesicula seminalis, which is large and flask-shaped, and this after coming to a quite narrow neck widens into the ductus ejaculatorius which is somewhat sausage-shaped and muscular, this terminates in a fine duct or tube which enters at its base a ball-shaped muscular structure the bulbus ejaculatorius, and through the centre of which it passes to end in the base of the chitinous cirrus, which is .05 mm. in length by .020 in width, hollow and terminating at the tip in two short hooks.

The genital connection takes place where the two columns of vitellaria connect anteriorly; this scheme or connection is shown in Fig. 12a.

As will be seen from the above description, this worm differs very materially from *torpedinis*, although found together and under the same circumstances; therefore, it would appear to be a nov. sp., for which I propose the name of *Amphibdella flavolineatus*.

Measurements of *Amphibdella flavolineatus*.

Length	4.50 to 6.00 mm.
Width	1.00 mm.
Sucker disc, width	.34 mm.
Sucker disc, length	.20 mm.
Ovary, length	.25 mm.
Ovary, width	.08 mm.
Hooks on sucker disc, length	.10 mm.
Pharynx, diameter	.10 mm.
Cirrus, length	.05 mm.
Cirrus, width	.02 mm.
Testis, length	.13 mm.
Testis, width	.09 mm.
Egg, length	.03 mm.

Fig. 13

FIG. 13.—*PARAMPHISTOMUM PAPILLOSTOMUM*

Fig. 14

FIG. 14.—*DISTOMUM MACRORCHIS*

Paramphistomum papillostomum, nov. sp.

(Fig. 13)

Family *Paramphistomidae* (Fisch.).
Sub-family *Paramphistominae* (Fisch.).

Before the above nomenclature was finally settled the name of this genus had caused a considerable discussion. In 1788 a worm was discovered by Schrank which from its description seems to have been an amphistomum. He named it *Festucaria strigis*, having found it in an owl. Then in 1790 Abildgaard found a similar worm and called it *Strigea*. In 1801 Rudolphi arbitrarily ignored any previous names given to the genus and named it *Amphistomum macrocephalum*, and he also applied this name as the synonym of a great number of species reported by other observers. This apparently held until 1901 when Fischoeder, in an attempt to clear up the whole matter of name, called the genus *Paramphistomum*, family Paramphistomidae, subfamily Paramphistominae. He intended, it is presumed, that this name should include all of the former genera and species of the so-called Amphistomidae. Looss does not think that there was any necessity for such a sweeping change and would adhere to the name *Amphistomum*, and in this no doubt a great number of investigators will agree, although a considerable number, including many of those who think *Amphistomum* proper on account of long use would rather see the original name *Strigea* adhered to. In all up to 1889 von Linstow records twenty-eight species as being found by six investigators of whom Diesing described thirteen species and Cobbold six. These were generally found in the herbivora as the elephant, rhinoceros, horse, buffalo, deer, etc. Since then many forms have been found in birds, especially in the hawk and owl families. Only one Cephalopod, the squid, has been credited with being the host of an *Amphistomum*, namely *A. loliginis*. Only two species of turtles so far as known have harbored this worm, viz. *Chelonia mydas*, the green turtle, and *Chelonia imbricata*, the hawksbill turtle. The former is credited with two species thus far—*A. scleroporum*, Creplin and *A. spinulosum*, Looss. Creplin found it first in 1844, Braun mentions it in 1893 and 1899, also again in 1901. Looss in 1901 describes *C. mydas* as being the host of *A. spinulosum*. It was found in the colon of a green turtle on the Egyptian coast near Alexandria. In 1902 he mentions it again as being found in *C. mydas*. Shipley described it in 1905 as found in *C. mydas*.

On January 31, 1914, while making an autopsy on a very large *C. mydas* at the New York Aquarium an *Amphistomum* was found in the intestines. It was a solitary specimen, yellowish in color, thick and firm in structure and measured when fresh 10 mm. in length by 5 mm. in width. As this species of worm is not so frequently found in amphibians as in animals and birds, I therefore record its occurrence and describe it very briefly. (Fig. 13.)

The worm is oval and thick with skin smooth and unarmed, therefore I conclude that it is not *A. spinulosum;* in this it is very different except that it possibly might be that Looss each time had a young worm to describe, so also Shipley, while mine may have been a very old specimen which had lost its spines as is not unusual with some of these worms. As to its identity with Creplin's *A. sclerosporum*, I do not find that it resembles it at all, although evidently belonging to the same family. The most striking features of *P. papillostomum* are its mouth and posterior sucker. The opening of the mouth is terminal and somewhat oval, and measures 0.60 mm. from side to side. The lips or margin present a number of slight or short papillae or rod-shaped projections which, parallel to one another, entirely surround the opening. They measure in length 0.02 mm. Internally the mouth opens at once into a large globular bowl-shaped structure, occupying the whole anterior end of the worm, and in this differing from many worms of the genus which have long lateral suckers or ceca. At the lower edge of this and partly within it is the pharynx. The whole giving the impression that the sucking apparatus is very powerful. The pharynx is somewhat round. There is no pre-pharyngeal oesophagus, but the post-pharyngeal oesophagus is relatively long, being 1 mm. in length before it divides into the cecal rami. These latter proceed posteriorly almost to the caudal sucker, and although much lobulated, do not give off any branches. In the middle of the angle where the ceca separate is seen the genital pore at which the mouth or anterior end of the uterus and cirrus appear, coming in different directions from behind the large testes. On account of the testes covering in the vas deferens and cirrus to so great an extent—that is, when viewing the worm from the ventral aspect—the exact structure of the cirrus cannot be made out, nor does the metraterm appear. The testes, which are two, large more or less square blocks, occupying fully half the width of the body and filling the whole space between the ceca in that region, are placed almost immediately behind the genital pore and almost in contact with one another.

The ovary is roundish, fairly large and situated on the left side somewhat, and at the junction of the anterior two-thirds of the body with the posterior third, and here, too, is the genital junction where the vitelline ducts join the oviduct and near which the shell gland is situated. The uterus coils several times from side to side across the middle of the body and is well filled with eggs which are round and yellowish in color and measure 0.02 mm. in diameter. No seminal reservoir is seen. In the centre of the body towards its caudal sucker is a large opening, the excretory pore, but the water vascular system is not much in evidence although, of course, present. The vitellaria occur in large rounded masses and they occupy, in a scattered way, both sides of the body throughout its posterior half. A very striking feature is the caudal sucker which is sub-terminal. It is pyriform in shape, base anteriorly, large and exceedingly muscular. The opening

in it is also quite pyriform in shape, the small end being posterior. This is apparently the sole means of adhesion to its host.

As far as I can make out *P. papillostomum* does not quite fit into the description given of either *A. spinulosum* (Looss) or *A. scleroporum* (Creplin). The size of *A. spinulosum* is given as 5 to 5.6 mm. in length, about half the length of *P. papillostomum*. The conformation of the internal organs does not correspond nor is the skin at all like it since there is not a spine to be found on it, neither are the circular rugae or papillae seen described as the base of these spines, nor are there any spines about the mouth. In the present worm the skin is perfectly smooth, although somewhat wrinkled, but there are no signs of papillae. If it be said that young worms often show spicules on the skin which later they lose, that probably would not apply to the circular rugae or the papillae which support the spicules. The skin is traversed from end to end by fine striae, which give it the appearance of having been sectioned by a knife with a very imperfect or finely notched edge. This is evidently owing to the muscular fibres in the skin and anteriorly cross fibres show. The mouth is very different, being surrounded by a row of very regular small papillae placed side by side, forming the lips, and no doubt they answer the purpose of feelers as well as assisting in the process of sucking. The same objections would be called up in considering the resemblance to *A. scleroporum*. The general appearance, the subterminal mouth, the round caudal sucker and narrow slit-like opening, not to speak of the internal conformation of the organs. As there have been so many species described, I feel very reluctant to add to their number, and yet there does not seem to be any proper form hitherto described in which as a species it could associate or be classed.

Measurements of *Paramphistomum papillostomum*, nov. sp.

Length	10.00 mm.
Width	5.00 mm.
Length of caudal sucker	2.20 mm.
Width of caudal sucker	2.00 mm.
Length of opening in caudal sucker	1.40 mm.
Width at base of caudal sucker	1.20 mm.
Mouth from side to side	0.60 mm.
Length of papillae of lips at mouth	0.02 mm.
Testes length	2.00 mm.
Testes width	1.00 mm.
Eggs, diameter	0.02 mm.
Length of oesophagus	1.00 mm.

Distomum macrorhinis, nov. sp.

(Fig. 14)

During the examination of an elephant seal, *Macrorhinus angustirostris*, on February 29, 1912, at the New York Aquarium, there were found a large number of little distomes in the intestine. They were not restricted for room for the intestine was twenty-three and a half metres in length and the only other inhabitants of this tract were a few ascarides. These were from 35 to 50 mm. in length.

The little distomes are 1.40 x 0.40 mm. and the anatomy is exceedingly simple. The anterior sucker is subterminal and occupies almost the entire anterior end. Leading from this is a short prepharyngeal oesophagus. The pharynx is not large nor is there anything peculiar about its structure, it ends in a postpharyngeal oesophagus which is very long, being at least eight times as long as the portion in front of the pharynx. At about a fifth of the length of the worm it divides into the ceca which extend to near the middle of the worm before they become hidden by the vitellaria, after which they cannot be followed but probably extend to nearly the posterior end. There is plainly visible beginning at each side of the anterior sucker a water vascular tube which extends also along the sides of the body to be lost under the vitellaria, but to terminate in the large excretory pore.

The genital organs are situated mainly towards the extreme posterior region. The female genitalia consist, as usual, of an ovary which in this case is an irregularly elongated structure which gives off the oviduct at about the middle of the body in the posterior third and this almost at once receives the duct from the seminal reservoir and the vitelline duct, and these, with the shell gland, form the genital junction. The uterus from near here winds in tubular folds loaded with eggs across from side to side several times, and finally terminates in the genital pore situated at the anterior edge of the acetabulum. Eggs measure 0.015. The vitellaria are very plentiful and extend from a short distance in front of the acetabulum and fills in most of the posterior two-thirds of the worm.

The male genitalia consist of two triangular-shaped testes situated very near the posterior end of the body cavity. They are relatively large and each gives off a vas which join to form the vas deferens which almost at once widens into a fairly large vesicula seminalis. From this the vas deferens ends in the cirrus at the genital pore.

The skin of the worm is not smooth, being thrown into wrinkles, but it is unarmed. The acetabulum is very large compared with the mouth sucker, being probably four times as large. It is situated a little posterior to the middle of the worm. Other than the oesophagus and the water vascular tubes which are somewhat branched almost the whole anterior half of the body is unoccupied with any important structures, except part of the alimentary canal and the water vascular system.

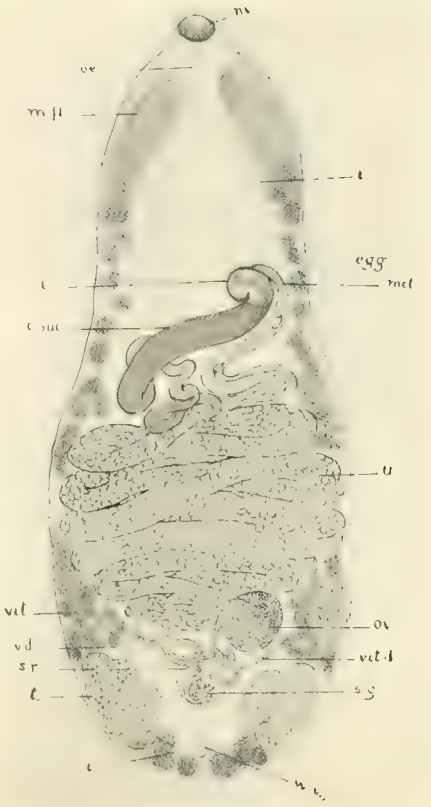

Fig. 15

FIG. 15. *MONOSTOMUM POMACANTHI*

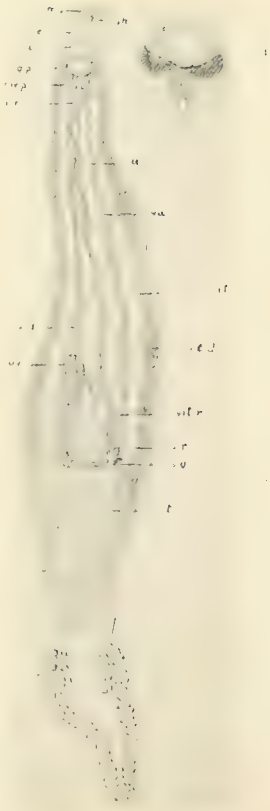

Fig. 16

FIG. 16. *MICROCOTYLE ANGELICHTHYS TOWNSENDI*
t, Head-gland.

Monostomum pomacanthi, nov. sp.

(Fig. 15)

On May 29, 1915, while examining a large French angel fish—*Pomacanthus paru*, from the New York Aquarium—there was found a number of *Monostoma* in the intestines. They are about 4 mm. long by 1.02 wide, are elongated, quite pointed at the head and rounded at the posterior end. The most prominent feature of the body, even as seen with the naked eye, is the peculiar clasping muscular flap on each side anteriorly, apparently intended by nature to assist the worm in keeping itself in position, or possibly in conjugation, as in the case of *Bilharzia hematobium*. The muscular fibers are very distinct, and interlace in all directions, concentrating at the point of the flap. The mouth is large and apparently strong. The oesophagus is indistinctly defined and extends one-eighth the length of the worm before it divides into the ceca. No pharynx is seen. The muscular flaps start on each side well forward in the neck, and almost at once widen so as nearly to meet in the centre of the body, then they gradually slope away, disappearing at each side at a point nearly one-half the length of the worm. In this enclosed space the oesophagus and ceca lay, and in its posterior area is the genital pore, where the uterus and cirrus terminate. The uterus is voluminous, and filled with oval eggs which each have a long chitinous filament attached to either pole. The uterus is provided with a strong metraterm near the genital pore. The vas deferens after passing behind the folds of the uterus appears anteriorly to them in a relatively large cirrus sac, this coils towards the genital pore, which is near the side of the worm. The pars prostatica is well developed, and the whole sac is well supplied with muscular tissue.

The testes are two in number situated almost at the extreme posterior end of the body, one on each side. They are much larger than the ovary, which lies in front of and between them, and here also is the genital junction. The vitellaria is distributed in the form of two large lobulated masses at the sides in front of the testes, although in some specimens masses may be seen along the sides of the body elsewhere, but generally the vitellaria are as described in front of the testes. The water vascular system drains the body from the head and terminates in a large oblong opening very near the posterior end of the body. The nervous system is not very evident. A lateral view of the worm shows it with rounded shoulders, and the flaps extending in front, almost like two short arms.

Measurements of *Monostomum pomacanthi*, nov. sp.

Length	6.00 mm
Width	2.00 mm.
Mouth	.025 mm.
Body across claspers	1.04 mm.

Microcotyle angelichthys townsendi, nov. sp.

(Fig. 16)

This worm, like the rest of the genus, inhabits the gills of its host which in this case was the yellow-tailed angel fish, *Angelichthys townsendi*. It is small, being only 3.05 mm. long and 0.20 mm. wide. It has a short almost straight sucker disc, coming to a sharp point which to the naked eye gives the worm the appearance of being sharp at both ends and the whole body but slightly bent still fairly thick. Only about twenty specimens were washed from the gills in the case examined. The fish was small, being only six inches by three inches, yet the worms were hardly in sufficient numbers to cause death. The head is roughly triangular, at least the most anterior portion is prolonged to almost a point, and this is provided with three tactile areas. The mouth suckers are round cuplike and appear very muscular. They are somewhat peculiar and quite different from those of most members of this genus. The two suckers are placed obliquely in the throat, and slanting from before downwards and backwards, each sucker seems to be divided in two by a slight band, and they are otherwise marked by striae, probably muscular, which run from the centre to the edge of the cuplike sucker. Anterior to these suckers in the centre of the mouth the edge is prolonged into a sort of proboscis and used probably to hold firmly the gill filament upon which it is feeding. On each side of this proboscis is another small sucker which probably has the same function as the proboscis. Behind these the pharynx is very near and is also round. More posteriorly still is the genital pore or atrium which differs distinctly from that of any of the other members of the genus and is the peculiarity of this species. The atrium is divided into two openings, the posterior one being that for the cirrus which is generally seen protruding a short distance over the edge. It is surrounded by hair-like spicules, and this opening is separated from the uterine opening superficially by a narrow band of spicules, while it can also be seen that the openings are separated by a membrane.

The uterine opening is profusely surrounded by long hair-like spicules especially in front or anterior to the pore; here the armature is in a wide dense mass. The whole genital outlet occupies almost the whole width of the worm, indeed the body is distinctly widened at this part to accommodate these structures.

The uterus and vas deferens proceed anteriorly side by side along the middle of the worm until they reach near to the atrium, the uterus generally lying along the left side of the vas throughout its course, but here it becomes more superficial ventrally and curls over the cirrus pouch to the right to make its way to the uterine outlet.

The vagina opens dorsally and centrally at a point nearly one-third the length of the worm from the head. It is relatively large and soon divides into two ducts to join the vitelline ducts or reservoir.

The ovary is placed posterior to the middle of the body and is loosely distributed in the shape of a horse-shoe. The looseness of the structure is not due to postmortem pressure, as great care was taken to prevent that. Generally as the worm lies on its back the end of the ovary on the right side gives off the oviduct which in a winding way proceeds posteriorly and over to the left side, enclosing in its folds the seminal reservoir and proceeding to the shell gland which is always in this worm particularly prominent and highly stained. On passing to the shell gland it receives the duct from the seminal reservoir and also the yolk ducts. On passing through the shell gland it curves anteriorly and proceeds along the middle of the worm to the atrium.

The vitellaria are unusually profuse extending along each side from almost opposite to the mouth to the disc posteriorly.

The testes, about twenty-six in number, are fairly large and of a roundish shape and extend from near the disc nearly up to the ovary.

On each side of the body and between the vitellaria and the internal organs along the middle of the body are two lines distinctly free of vitellaria, making a stripe along almost the whole length of the body. These lines are plainly traceable into the sucker disc and are no doubt the site of the water vascular system, although no vessels could be seen. The disc is distinctly foot-like in an extended position, but is very often held in a perfectly straight position. It is short and supports about fifty-six clasps or suckers. These are slightly different in shape from those in other species, being wider in their grasp and not so deep, although their chitinous structure is much the same.

Measurements of *Microcotyle angelichthys townsendi.*

Length	3.05 mm.
Width	.15 mm.
Disc length	.20 mm.

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A STUDY OF THE INVOLUTIONAL CHANGES WHICH OCCUR IN THE ADRENAL CORTEX DURING INFANCY.*

R. W. Lewis, M.D., and A.M. Pappenheimer, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons Columbia University, and from the Department of Pathology of the New York Nursery and Child's Hospital.)

The extraordinary degenerative changes which occur in the adrenal cortex during the earliest period of infancy have attracted surprisingly little attention since their first description by Thomas[1] in 1911. Indeed, not much of importance has been added to his observations, and their significance remains entirely obscure.

Thomas[1] discovered the fact, curiously overlooked or misinterpreted up to that time, that during the first year of life there regularly takes place a complete degeneration of the inner zone of the cortex. He describes, as examples of this process, the adrenals of four infants aged one, three, six, and twelve months respectively. In the child of one month, the cells of the inner zone of the cortex were found in all stages of nuclear and protoplasmic degeneration. There was great vascularity of the tissue, with some extravasation of blood. In the second case (2 months), the superficial portion of the cortex was unchanged; the deeper portion showed even more advanced degeneration of the cells; in some areas, especially where the growing medullary tissue lay in the vicinity of the intact cortex, the tissue was composed wholly of lamellæ of fibrous tissue, with cell detritus in its meshes. In the third case (6 months), the degenerated zone contained few intact cells and was represented by closely packed layers of connective tissue. The medulla was noticeably increased in amount. In the fourth case (12 months), a narrow strip of vascular connective tissue separating the broad medulla from the intact cortex, occupied the original place of the degenerated zone. Between the fibrils it was still possible to find cell detritus, and in places intact red cells and free pigment.

The interpretation which Thomas places upon this process is that the cortex degenerates to make room for the growing medulla, which is able to encroach upon the cortical tissue only because it is partially degenerated and non-resistant. As to the influence of the intense hyperemia always found at birth in the inner cortical zone upon this degeneration, no conclusions could be drawn. It seemed, however, that the small hemorrhages which occur regularly at birth might exert a harmful

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influence upon the cortical cells, since these undergo pigmentary degeneration after engulfing the red corpuscles.

No normal cases were studied. Nevertheless, it was thought to be extremely unlikely that the cortical changes depended upon general disease processes because of the fact that identical changes were associated with such widely different diseases as lues, diphtheria, marasmus, sepsis, etc.

Elliott and Armour,[2] independently of Thomas' observations, only a few months later described similar alterations in the adrenal cortex of infants after birth. To quote the summary of their findings: "The large size of the human suprarenal gland during fetal life is due to a peculiar hypertrophy of the cortex which commences early and continues until birth. The inner hypertrophied mass is richly supplied with blood vessels; but it does not store in its cells the characteristic doubly refracting substance, which is a chief secretion product of the cortex. Immediately after birth, the inner mass of hypertrophied cells degenerated, undergoing fatty change; and at the end of the first year all trace of it has disappeared. Hemorrhages occur readily in this degeneration zone, which in consequence has been wrongly depicted in some text-books as medulla damaged by hemorrhage. Enveloping the mass is a rim of smaller cells that early in fetal life assume the appearance of the cells of the adult cortex and store up fatty substance. These develop steadily and alone form the adult cortex."

Kern,[3] almost coincidently with the appearance of the paper by Elliott and Armour, published a somewhat more detailed description of the same process. He divided the degenerative changes into four stages. The first stage, that of hyperemia, is that found in the new-born. The great filling of the capillaries loosens the columnar alignment of the cortical cells and produces displacements and compressions. Very early degenerative changes are evident in the shrinkage of some of the nuclei, and in the appearance of colloid droplets in the cytoplasm.

The second stage, that of completely established degeneration, is seen in the adrenals at the end of the first month, and lasts for a variable period reaching towards the end of the first year. The degenerating zone is sharply marked out from the intact superficial cortex, as well as from the medullary tissue. The degenerative changes are shown in the appearance of large fat droplets, colloid globules, and, in some cells, of peculiar fibrillary changes in the protoplasm, which he believed to indicate a production of connective tissue fibrils. The degenerating cells frequently appear as if flattened out, and come to lie with their long diameter parallel to the surface. Other features are phagocytosis of red blood cells, and thickening of the reticulum between the disintegrating cortical cells.

The third phase, that of the formation of a definite fibrous capsule about the medullary tissue, is usually completed at the end of the first year. The progressive disappearance of the degenerating cells, the diminishing hyperemia, and the real or apparent increase of the connective tissue scaffolding are the characteristic features of this stage. The

connective tissue, in the meshes of which are still found degenerating and pigmented cortical cells, is definitely arranged in the long axis of the gland.

The final phase of the process stretches from about the end of the first year to the completion of growth. The strip of connective tissue, which separates intact cortex from medulla, becomes progressively narrowed by the growing medullary tissue, and remains permanently only where the two layers of cortex are face to face.

Kern is certain that these changes are physiological and in no wise dependent upon pathological influences. He has, however, no explanation to offer as to their significance. Studies of the adrenals of guinea-pigs, rabbits, calves, oxen, pigs, sheep, rats, and horses failed to show post-natal involutional changes such as are seen in the human adrenal. Dewitzky [4] has also been unable to detect similar changes in the adrenals of animals.

The foregoing citations, together with brief references in some of the newer text-books, such as that of Brüning and Schwalbe,[5] comprise practically all that is known of this striking phenomenon. It seemed of interest, therefore, to follow somewhat more closely and in a larger series of cases than has hitherto been studied the sequence of changes; to determine the range of individual variation at different ages; and to see whether these variations could be correlated with pathological factors such as inanition or infection. Like the previous workers, we have lacked normal control material, which is obviously difficult to procure at that age.

Methods and material. — Our study is based upon the adrenals of a hundred unselected cases, ranging in age from the fifth month of fetal life up to three years. As we were concerned less with the minute histological changes than with the general phases of the involutional process, routine autopsy sections of material fixed in Zenker's fluid and stained with hematoxylin-eosin amply met the purposes of our study.

From each section a diagram was drawn with the Edinger projectoscope at a constant magnification of about nine diameters. This diagram indicates (i) the intact cortical zone; (ii) the zone of degenerating cortical tissue; (iii) the fibrous zone of replacement; (iv) the glomerular zone,

which under the power employed could be differentiated only in the adrenal of the new-born; (v) the medullary tissue. These different components are represented in the diagrams as follows: Intact cortex — unshaded; degenerating zone — cross-hatched. Where the degeneration zone is partially replaced by fibrous tissue, the cross-hatched area is enclosed by a broken line. When the degeneration is more advanced, so that the fibrous replacement is practically complete, the cross-hatched area is surrounded by a continuous line. Medullary tissue is indicated by solid black, the glomerular zone by stippling.

Measurements of the intact cortex and of the inner zones were made directly from drawings. To compensate for inequalities at different levels, an average of twelve measurements of each zone was taken for charting. The curves, with the ordinate indicating average width and the abscissa the age, are based on these measurements.

Several sources of error suggest themselves, which may perhaps detract from the value of this method. Measurements made from blocks cut obliquely through the gland may give too high figures. Edema likewise is a disturbing factor, but only in the glands of macerated still-births. In a certain percentage of the cases the age could be only approximately determined, since the subjects were foundlings. A further source of possible error lay in the difficulty in differentiating sharply the different zones, especially under the low magnification used; although in doubtful cases the drawings were controlled by study with the high power.

In spite of these possible objections which make the method rather a rough one, the data obtained probably afford a fairly satisfactory graphic record of the changes as they occur at different ages. The number of cases studied seems sufficiently large to overcome variations due to technical defects in the method.

We have purposely omitted reference to the medullary changes, since serial sections would be required to obtain an adequate idea of the amount and distribution of the medullary tissue. The medulla in the infant's adrenal is restricted

to a relatively small area, so that it does not appear at all in some of the sections studied.

General discussion. — The general course of the involutionary process is illustrated in Figures 1 to 6. With minor differences we have found the histological changes to take place very much as they have been pictured by Thomas and Kern, so that a detailed description seems superfluous. As is evident from the diagrams, and still more strikingly from the chart, there occurs within the first two weeks an extraordinary reduction in the width of the intact cortex. Even during the first few days, degenerative changes in individual cells may be found under the microscope, but a massive degeneration of the inner zone and its definite sequestration from the healthy outer tissue does not become manifest until the second week.

From this time on, up to the age of three years, where our observations cease, there is no appreciable increase or diminution in the breadth of the intact cortex.

The zone of degenerating tissue for the first few weeks varies inversely with the width of the intact cortex. After the fifth or sixth week the area occupied by the degenerating cells rapidly decreases; and with the removal of the cells and the collapse of the framework, the fibrous zone becomes clearly defined. From this time on there is a shrinkage of the fibrous zone, which after the fourth month progresses with extreme slowness. Neither our drawings nor our measurements indicate that there is developed a new-formed connective tissue capsule about the medulla, as Kern maintains. The shrinkage, on the other hand, may be accounted for by the diminishing vascularity, the more complete removal of the degenerating cells, and the progressive aging of the fibrillar stroma, and possibly by its compression by the growing medulla.

Individual variations. — The variations which we have observed fall into two groups: those in which measurement of the cortex shows a marked departure from the average,

but in which the stage of involution is appropriate for the age; and those in which involution is either hastened or retarded as compared with other glands of the same age.

The first type of variation is illustrated in Figures 7 and 8, representing glands from infants of approximately identical age, namely, one month and twenty-two days and two months and six days, respectively. The smaller gland was taken from the slightly smaller infant, the difference in length being four centimeters. It does not seem as if this marked variation could be correlated with the difference in the size of the infants. Both children were poorly nourished.

More frequently encountered were variations in development at a given age, and with this, often but not always, variations in the width of the intact cortex and of the inner zone. This type of variation is well shown by comparing Figures 9 and 10. The glands were taken from infants of the same age, eight and one-half months, each much emaciated. Again the smaller gland was obtained from the smaller infant, there being a difference of four centimeters in length. The smaller gland, however, shows a more advanced stage of involution, the fibrous capsule being completed as it normally should be at this age, whereas in the other gland the broad zone of degenerating tissue indicates a distinct retardation of involution. There is also marked difference in the width of the intact cortex.

Another striking instance of delayed involution is given in Figure 11, representing a gland from an infant of two months and thirteen days, which may be compared with Figure 12 from an adrenal of the same age (two months and twelve days). Although the cortical measurements are almost identical, the involuting zone in the first gland shows very numerous partially degenerated cells, with great congestion of the capillaries, whereas in the normally involuted gland the collapse of the tissue with the appearance of a distinct fibrous structure is well under way.

Two of the most striking examples of delayed involution were in infants which were definitely syphilitic. The first of these (Fig. 13), from a well-nourished baby of twenty-five

days, showed an intact cortex such as one might expect to find immediately after birth. The second, a child of eleven and one-half months, with a strongly positive Wasserman reaction, but no very definite syphilitic lesions at autopsy, also showed a somewhat delayed involution, there being many isolated degenerating cells still present in the inner cortical zone.

Our material has been insufficient to determine whether congenital syphilis is frequently responsible for this retardation of involution. In view of the rôle which syphilis is assumed to play in inhibiting the differentiation of other tissues such as thymus and pancreas, the possibility deserves further study.

In five other cases the lesions found at autopsy threw no light upon the cause of the retarded involution.

Examples of premature involution were less frequent, only two of the hundred glands examined being interpreted in this way. Figure 14 shows a gland of a three months infant in which the development of the fibrous zone is practically completed. The usual condition is illustrated in Figure 15, also from a child of three months. There seemed no obvious reason for the disparity.

Influence of prematurity. — Eight of the glands were from premature children. Although three of these show intact cortices which are thinner than the average, one may say that involution in general proceeds normally. This is of particular interest in that the changes which occur apparently depend upon the altered conditions of extra-uterine life, and seem not to be correlated with the development of any other organ or tissue. Were there such a correlation, one would expect the involution of the adrenal to be halted until that organ had attained the stage of development normally reached at birth.

Influence of infections. — Although normal control cases were lacking, it seemed as if some information as to the influence of infection upon the involution could be gained

by comparing the changes found in cases of pneumonia with those in which no evidence of general infection was recognized at the autopsy.

The data bearing upon this point are summarized in Table I.

TABLE I.

Age.	Pneumonia Cases.			Non-infectious Cases.		
	No. of Cases.	Average Width Cortex.	Average Width Inner Zone.	No. of Cases.	Average Width Cortex.	Average Width Inner Zone.
11 hours to 5 days . . .	3	14.9	0	4	13.9	0
28 days to 1 month 1 day	4	4.0	3.7	2	4.0	4.1
2 months to 2 months 8 days	2	5.2	1.7	2	3.7	1.8
2 months 9 days to 2 months 13 days . . .	3	5.6	2.8	1	3.4	2.3
2 months 29 days to 3 months 3 days . . .	3	4.0	3.0	4	3.6	2.2
7 months and 6 months 20 days	1	3.9	0.9	1	4.1	1.4
8 months 10 days to 8 months 17 days . . .	4	4.2	0.8	1	2.6	1.1
8 months 23 days to 9 months	2	4.8	1.9	1	3.4	0.7
10 months 28 days and 10 months 29 days .	1	6.4	0.6	1	4.2	0.7
13 months and 13 months 24 days . . .	1	5.2	1.0	1	4.9	0.7

The interesting fact is brought out that with one exception (cases of seven months and six months and twenty days) the width of the intact cortex in the pneumonic cases is equal to, or more commonly in excess of, that in the non-infectious cases. The cause of this difference is not clear. Many of the glands are hyperemic and a few are edematous, but it is not evident how edema or hyperemia can lead to an increase in the width of the cortex, although they might very well cause lateral compression of the columns of cortical cells.

One might indeed have expected the cortex in the pneumonic cases to be narrower, because of the marked depletion

of lipoids which is found in pneumonia (Weltmann,[7] T. Elliott[6]), and the consequent shrinkage of the cells.

Aside from this clear difference in the width, no influence of the infection upon the process of involution is manifest.

Influence of nutrition. — A glance at Chart II., in which the glands from emaciated cases are compared with those of the well-nourished, shows that inanition does not modify in any way the course of the involutionary process. The two curves overlap at various points, but in general they coincide within the normal limit of variation. It does appear as if involution began somewhat later in the well-nourished infants, but this is because the measurements include the case of the syphilitic infant of twenty-five days described above, in which there was a decided retardation.

Involution in accessory or aberrant cortical tissue. — In the small encapsulated masses of cortical tissue partly embedded in the adrenal, or lying in the adjacent fat and connective tissue, the involutional changes take place as in the main gland. This is of interest as indicating the effect of some general agent acting through the blood stream, rather than of local conditions within the gland itself. It effectually disproves the idea that the development of the medullary tissue is directly responsible for the changes in the cortex.

CONCLUSIONS.

Our studies, like those of previous workers, give no clue to the inner significance of the post-natal degeneration of the cortex. They do, however, bring out clearly certain features, which may perhaps be more intelligible when the meaning of the whole process is better understood.

(1.) The degeneration is practically accomplished at the end of the second week; although individual degenerating cells are found for a long time, the abrupt fall in the width of the cortex during the second week can only mean that the great mass of cells is destroyed at that time.

(2.) There is no regeneration of cortical tissue during the first three years of life.

(3.) The fibrous zone between medulla and cortex is formed simply by the collapse of the framework, and there does not appear to be a new formation of fibrous tissue.

(4.) Individual cases may show either acceleration or retardation of the involutionary process. For the latter, syphilis is suggested as a possible cause.

(5.) Neither prematurity nor inanition noticeably modify the course of involution.

(6.) Infection, as exemplified by pneumonia, seems to be associated with an increase in the width of the intact cortex.

[NOTE. — Since this paper has gone to press there has reached us a monograph by M. Landau (Die Nebennierenkinde, Jena, 1915, G. Fischer) which includes a study of eighty-seven adrenals of children from birth up to ten years of age. The general description of the involutional changes coincides with that of Thomas, Elliott, and Kern, and no relation to special disease conditions or to the development of other ductless glands was found.

The purpose of the cortical degeneration, according to Landau, is to increase the infolding or convolution of the surface, and, in consequence, to enlarge the area of contact between cortex and medulla. Only general arguments are advanced in support of this view, and the immediate cause of the cortical destruction in post-fetal life is left unexplained.]

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7. Weltmann, O. Ueber das doppeltbrechende Lipoid der Nebenniere. Ziegler's Beitr., 1913, lvi, 278

CHART I.

Curve of Involution in 100 Unselected Cases.

Width of intact cortex — continuous line.
Width of inner zones — interrupted line.

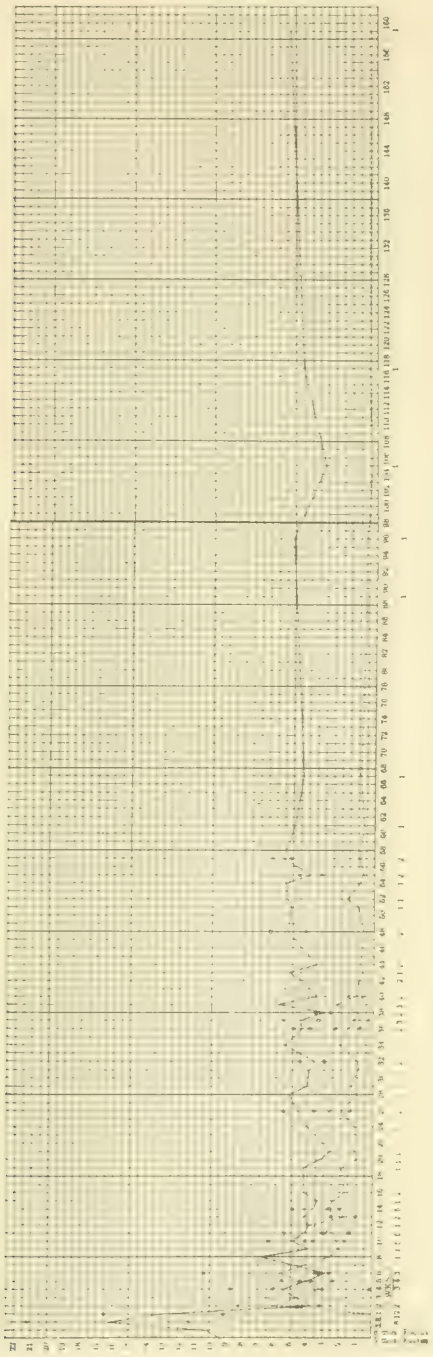
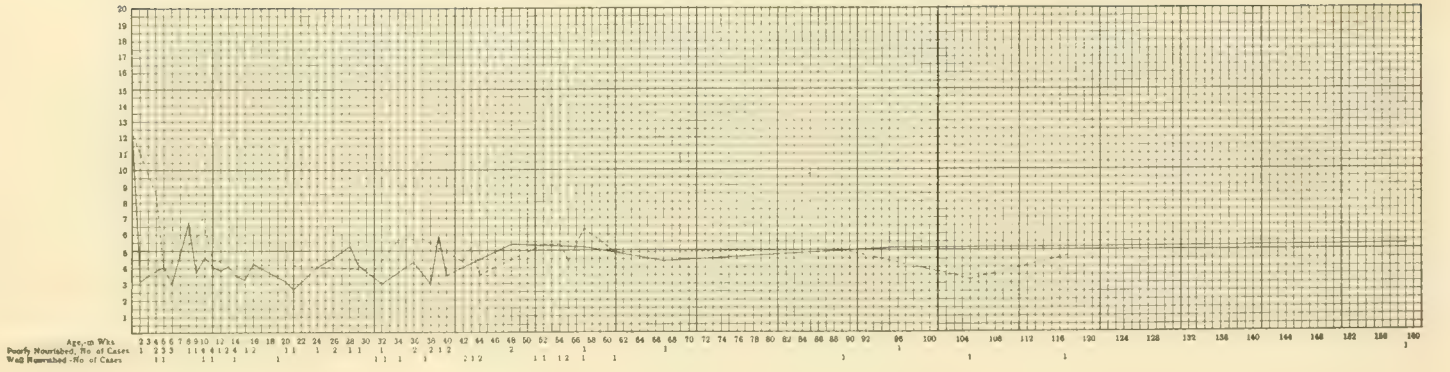


Chart II.

Influence of Nutrition upon Involution.

Poorly nourished cases — continuous line.
Well nourished cases — interrupted line.



DESCRIPTION OF PLATES IV. AND V.

FIG. 1. — Still-birth.
FIG. 2. — Five days.
FIG. 3. — Eleven days.
FIG. 4. — One month and six days.
FIG. 5. — Four months and seventeen days.
FIG. 6. — Ten months and twenty-eight days.
FIG. 7. — One month and twenty-two days.
FIG. 8. — Two months and six days.
FIG. 9. — Eight months and sixteen days.
FIG. 10. — Eight months and twenty-three days.
FIG. 11. — Two months and thirteen days.
FIG. 12. — Two months and twelve days.
FIG. 13. — Twenty-five days.
FIG. 14. — Two months and twenty-nine days.
FIG. 15. — Two months and thirty days.

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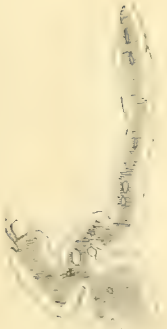
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TECHNIQUE OF CULTIVATING HUMAN TISSUES IN VITRO.

By ROBERT A. LAMBERT, M.D.

(*From the Pathological Laboratory of the Presbyterian Hospital, Columbia University, New York.*)

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In 1910 Carrel and Burrows[1] reported the successful cultivation *in vitro* of a human sarcoma. They used as a culture medium plasma from the patient from whom the tumor was removed, and observed an active migration of cells during several days' incubation. No subcultures were made. In subsequent attempts to cultivate human tissue Carrel[2] experienced considerable difficulty owing apparently to the liquefaction of the clotted plasma medium. He noted, as did also Lambert and Hanes,[3] Maccabruni,[4] and others, that within 24 hours a clear liquefaction zone appears around each tissue fragment, and that after a few days the entire fibrin clot becomes liquefied. Unless the cells wander out early they find no framework upon which to grow.

Losee and Ebeling[5, 6] attempted various modifications of human plasma with the object of preventing liquefaction, but were unsuccessful. However, by diluting the plasma with Ringer's solution, which seemed to delay digestion, and by making transfers of the tissue fragment to fresh plasma every 24 to 48 hours, they were able in a few instances to propagate human connective tissue cells obtained from fetuses through a number of subcultures, in one case as long as 60 days. They attributed their success in part to the addition of tissue extracts to the medium, as recommended by Carrel.[7] They emphasized the shortcomings of

[1] Carrel, A., and Burrows, M. T., Human Sarcoma Cultivated outside of the Body, *J. Am. Med. Assn.*, 1910, lv, 1732.

[2] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.

[3] Lambert, R. A., and Hanes, F. M., The Cultivation of Tissue in Plasma from Alien Species, *J. Exp. Med.*, 1911, xiv, 129.

[4] Maccabruni, F., Esperienzi di coltivazioni "in vitro" del cancro uterino umano, *Ann. ostet. e ginecol.*, 1914, xxxvi, pt. i, 57.

[5] Losee, J. R., and Ebeling, A. H., The Cultivation of Human Tissue *in Vitro*, *J. Exp. Med.*, 1914, xix, 593.

[6] Losee and Ebeling, The Cultivation of Human Sarcomatous Tissue *in Vitro*, *J. Exp. Med.*, 1914, xx, 140.

[7] Carrel, Artificial Activation of the Growth *in Vitro* of Connective Tissue, *J. Exp. Med.*, 1913, xvii, 14.

their technique and stated that they experienced many failures, for which the early rapid liquefaction of the fibrin was held responsible.

In our first efforts to cultivate human tissues in which unmodified human plasma was used as a culture medium, the results were practically the same as those of Carrel, and Losee and Ebeling. In only exceptional cases were we able to obtain satisfactory growths. There was regularly rapid digestion of the fibrin about the pieces of tissue, thus completely blocking cell migration. We therefore undertook to overcome this difficulty. It may be noted here that it is not the ability of human tissue to digest fibrin, but rather the susceptibility of human fibrin to digestion which is the basis of the trouble, for we found that human tissue did not liquefy the fibrin of foreign plasmas, while it was observed that human fibrin was readily digested by the ferments of practically every foreign tissue.

In the cultivation of the tissues of a number of lower animals,—rats, mice, guinea pigs, rabbits, dogs, cats, fowls, and pigeons,—using the plasma from these and other species, liquefaction was not infrequently observed, though never so regularly or extensively as in cultures containing human plasma. It was noted, moreover, that fowl and pigeon plasma were never liquefied, except in the presence of particular types of bacteria. Since earlier experiments had shown that tissues from certain of the lower animals could be cultivated in plasma from foreign species,[3] it occurred to us that this property of fowl and pigeon plasma to resist digestion might be utilized in growing human tissues. It seemed possible that chick fibrin might be made to serve as the framework of the culture medium with human serum or plasma added to supply the necessary nutritive substances. A medium was therefore prepared by mixing a small quantity of chick plasma with a considerably larger quantity of human serum. Various human tissues (lymph gland, spleen, skin, etc.) obtained at operation were put up in this mixed medium. No liquefaction was observed, and active progressive growth with numerous mitotic figures was seen in the majority of the preparations where soft friable tissue was used. Tissues removed at autopsy several hours after death were cultivated, the preparations showing in some instances a very active growth of connective tissue cells. It was not necessary

to make transfers to fresh plasma oftener than every 5 days. Continued propagation through subcultures was carried on for several months with as little difficulty as in the cultivation of similar tissues from lower animals. It may be noted that human tissues will grow in pure fowl plasma, but growth is not so active or so prolonged as in the modified medium containing human serum. Various proportions of fowl plasma and human serum were tested with the object of working out an optimum medium. A mixture of chick plasma, one part, and human serum, four parts, seemed to yield the best growth. The use of a relatively small amount of plasma has also an economic advantage in that human serum may obviously be obtained more easily and in larger quantity than fowl plasma. Furthermore, serum is easily preserved, whereas plasma sometimes clots after a few hours, even when kept cold in paraffined receptacles. It may be suggested that a still smaller proportion of plasma can be used since one part of plasma to twenty of serum will form a fairly firm clot.

The human serum used in these preliminary experiments came from a single individual. Autogenous plasma was not used since studies upon the cultivation of the tissues of lower animals had shown that plasma from the animal supplying the tissue was no better as a culture medium than plasma obtained from other members of the same species. For example, it has been found that the cells of transplantable mouse and rat tumors would grow quite as well in the plasma of rats and mice artificially or naturally immune to these tumors as in the plasma of tumor-bearing animals.[8] It seemed conceivable, however, that human tissues might prove more sensitive to individual variations in the culture medium. While studying this question it occurred to us that the influence of naturally existing iso-antibodies (agglutinins and hemolysins) upon growth *in vitro* might be investigated at the same time.

The interagglutinating reactions of human bloods, due, as Landsteiner and Leiner[9] and others have shown, to the existence of two

[8] Lambert and Hanes, A Study of Cancer Immunity by the Method of Cultivating Tissues outside the Body, *J. Exp. Med.*, 1911, xiii, 505.

[9] Landsteiner, K., and Leiner, K., Ueber Isolysine und Isoagglutinine im menschlichen Blut, *Centr. Bakteriol., Ite Abt., Orig.*, 1905, xxxviii, 548.

agglutinins and two agglutinogens in four different combinations[10] are well known. By simple agglutination tests any individual is found to fall into one of four groups depending on the power of his serum to agglutinate the blood of certain other persons, and on the susceptibility of his blood to agglutination; that is, on the presence or absence of one or both of the two agglutinins and agglutinogens.

For our experiments serum was obtained from individuals belonging to each of the four groups, and from the patients whose tissues were used for cultivation. The following protocol is illustrative of the results obtained.

Experiment 210.—An axillary lymph gland, removed at operation and showing microscopically changes characteristic of Hodgkin's disease, was placed in a sterile dish of cold salt solution. 1 hour later tissue cultures were put up in each of the following sera, to which chick plasma was added in the proportion of one part of plasma to four of serum: Group I, Group II, Group III, Group IV, and patient's serum (Group II). Ten cultures of each series were prepared and examined daily during the following week of incubation. Within 24 hours active migration of large mononuclear wandering cells and leukocytes was noted in practically every culture. On the 2nd and 3rd days a beginning outgrowth of connective tissue cells was observed, reaching a maximum activity about the 5th day. At no time could any difference in the five series be detected. The usual variations in individual cultures were seen, but the average extent of outgrowth was practically the same for each series. There was likewise no difference in the frequency of mitotic figures, which can be readily recognized by the practised observer in the living unstained preparations.

Other experiments in which tissues from Group I and Group III individuals were used gave the same result. While we were unable to secure any tissue from a person belonging to Group IV, as such

[10] It is assumed that there exist in human sera two agglutinins, *a* and *b*, and two corresponding agglutinogens, *A* and *B*. The blood of Group I possesses both agglutinins but no agglutinogens. It therefore agglutinates the blood of all other groups, but is not agglutinated by any serum. Group II possesses agglutinin *a* and agglutinogen *B*. It agglutinates blood of Group III and Group IV, and is agglutinated by members of Group I and Group III. Group III, the reciprocal of Group II, possessing agglutinin *b* and agglutinogen *A*, is agglutinated by Group I and Group II sera, and agglutinates the corpuscles of the third and fourth groups. Group IV contains no agglutinin but has both agglutinogens. Its serum therefore has no agglutinating power, but its corpuscles are agglutinated by the sera of all other groups.

people are rare, we feel justified in concluding that normally existing iso-antibodies in human blood do not influence in any way the growth of human tissues *in vitro*.

In addition to the question of culture medium we have been concerned with another problem in connection with the technique of cultivating human tissues *in vitro;* namely, the preservation of tissues after removal from the body. It is often not convenient to prepare cultures immediately upon the receipt of the tissue from the operating room or autopsy table. It is also desirable in many cases to carry out a number of experiments with the same tissue, not possible in a few hours' time. Furthermore, the duration of life of tissues in cold storage is a question of biological interest as well as of practical importance. In order to determine the period of survival under conditions of ordinary ice box preservation, cultures of the tissue were made on the day of removal, and at 2 day intervals thereafter, up to 12 days. A type experiment is described in the following protocol.

Experiment 320.—A piece of small tumor removed surgically from the region of the parotid gland (microscopically, a typical mixed tumor) was placed immediately in the ice box in a dish of physiological salt solution (0.8 per cent). Half an hour later the tumor, which was composed of soft friable tissue, rather gelatinous in places, was cut into small pieces. From some of these, cultures were made immediately. The remainder, covered with salt solution in a Petri dish, were returned to the ice box, an ordinary, small wooden structure with a lid, the temperature of which fluctuated between 10° and 15°C. The ten cultures put up on the day of operation grew well, showing after 2 to 3 days' incubation numerous large cells of irregular shape with frequent mitotic figures. Cultures made on the 2nd and 4th days of preservation, showed similar activity, though in the case of the tissue preserved for the longer time there was a longer latent period before migration was observed. Tissue kept for 6 days showed growth in the majority of preparations, though not so good as those put up on the 4th day. In the 8 day series only a few active cells were seen. Preparations of tissue kept for 10 and 12 days were all negative.

In another experiment in which a piece of lymph gland was used, no cells survived longer than 6 days. A glioma showed some growth after 8 days' preservation, but none after 10 days.

These experiments show that under very simple conditions of preservation human connective tissue cells and the cells of certain benign

tumors remain alive in the ice box for 6 to 8 days at least. 10 days probably represents the limit of survival. The period of survival is approximately the same as that determined for normal rat connective tissue, though somewhat shorter than that for embryonic chick tissues, which previous experiments have shown live 8 to 16 days in storage.[11] The optimum temperature for chick tissues was found to be about 6°C. The optimum temperature for preserving human tissues was not determined.

CONCLUSIONS.

1. Unmodified human plasma is not a satisfactory culture medium for human tissues owing to the susceptibility of human fibrin to digestion by tissue ferments. The necessary framework is thus destroyed before the cells begin to migrate. The difficulty can be overcome by adding to human plasma or serum a small quantity of fowl or pigeon plasma, the fibrin of which is highly resistant to digestion. Human tissues have been propagated in this medium for several months through subcultures, and growth *in vitro* can probably be maintained indefinitely.

2. Human tissues show no greater sensitiveness to changes in temperature and mechanical injury associated with preparation of cultures than those of lower animals. They may be preserved in an ordinary ice box at 10–15°C. as long as 6 or 8 days. Tissues obtained at operation give best results, but pieces of organs removed at autopsy 1 to 4 hours after death sometimes show active growth.

3. The presence of normally existing iso-antibodies (agglutinins and hemolysins) in human serum is without influence on the growth of human tissues *in vitro*. In other words, autogenous serum has no advantage in tissue cultures over homologous serum.

For the agglutination tests carried out in connection with the experiments reported, I am indebted to Miss M. P. Olmstead of the bacteriological laboratory of the Presbyterian Hospital.

[11] Lambert, R. A., The Influence of Temperature and Fluid Medium on the Survival of Embryonic Tissues *in Vitro*, *J. Exp. Med.*, 1913, xviii, 406.

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THE INFLUENCE OF AUTOLYSIS UPON CHOLESTEROL ESTERS.

By J. HOWARD MUELLER.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

(Received for publication, May 26, 1916.)

It has been shown[1] that cholesterol is absorbed into the animal body from the intestinal tract by way of the thoracic duct. No matter whether it is fed in the free condition or in the form of esters of the higher fatty acids, it is always found to have been converted to a mixture of about one part free to three or four parts ester by the time it reaches the chyle.

There are probably two quite distinct functions served by these two forms. Nothing can be said of the use of the esters of cholesterol, while in the free condition it is known to act at least *in vivo* as a neutralizing substance to a number of different poisons, such as saponin,[2] tetanolysin,[3] a hemolytic agent of the pneumococcus,[4] and also conceivably, poisons of metabolic origin. It seems desirable then, to study any further interchange between the two forms which may take place in the body.

A review of results reported by many different observers shows clearly that the total cholesterol of the blood serum of practically all species of animals reported upon (exclusive of human serum under pathological conditions) is always made up of both free and combined fractions, 25 to 35 per cent of the total being free. It will be noted that this is the proportion in which the cholesterol appears in the chyle after its absorption. When analyses of the red or white blood cells or of practically any

[1] Mueller, J. H., *J. Biol. Chem.*, 1915, xxii, 1.
[2] Ransom, F., *Deutsch. med. Woch.*, 1901, xxvii, 194.
[3] Noguchi, H., *Univ. Pennsylvania Med. Bull.*, 1902, xv, 327.
[4] Cole, R., *J. Exp. Med.*, 1914, xx, 346.

normal tissue, are made, the results are entirely different. In the red cells,[5] for example, there is probably no combined cholesterol; very little is found in the white cells, and the same is true for most other tissues. The brain contains practically no esters;[6] the kidney,[7] heart,[8] and liver contain varying proportions, from one-tenth to one-half of the total.

There are possibly two explanations for such a condition. Either the body cells are able by means of enzymes to split the esters of the blood plasma, and absorb the free cholesterol, or they absorb the free cholesterol selectively, and the esters are destroyed or altered in some way other than by simple hydrolysis. The first supposition seems the more reasonable, and experiments directed at the recognition of such enzymes have been carried out. It may be stated here that the results have been negative, but for reasons which will appear later it seemed worth while to present the material briefly at this stage.

The attempt has been made to show, by means of autolysis of blood, liver, and mixtures of the two, that there occurred hydrolysis of the esters of cholesterol which normally exist in such materials. This form of experiment and the selection of this material was suggested by two reports in the literature, and it was hoped to make these the basis for more extended work.

Schultz[9] autolyzed human, horse, and dog blood, and also liver and mixtures of blood and liver in the presence of sodium fluoride and thymol or chloroform as preservatives. He was unable to get any evidence of hydrolysis in blood alone, but in horse liver alone and in mixtures of horse, and, to a less extent, of sheep blood and liver, marked hydrolysis took place. Cytronberg,[10] in the same laboratory, reported positive results on horse and dog blood alone, and ascribed Schultz's failure to insufficient familiarity with the digitonin method of analysis. This work has

[5] Wacker, L., and Hueck, W., *Arch. exp. Path. u. Pharm.*, 1913, lxxiv, 416.

[6] Lapworth, A., *J. Path. and Bact.*, 1911, xv, 254.

[7] Windaus, A., *Z. physiol. Chem.*, 1910, lxv, 110.

[8] Hess-Thaysen, T. E., Habilitationsschrift, Copenhagen, 1913; abstracted in *Centr. Biochem. u. Biophysik.*, 1913–14, xvi, 85.

[9] Schultz, J. H., *Biochem. Z.*, 1912, xlii, 255.

[10] Cytronberg, S., *Biochem. Z.*, 1912, xlv, 281.

remained unconfirmed, except for the statement of Gardner and Lander[11] that in an autolyzed cat liver, no cholesterol esters could be found.

There are certain peculiarities about the figures quoted by Schultz and by Cytronberg which may be mentioned briefly. Both workers analyzed equal quantities of blood, usually 200 cc. before and after autolysis. Corper[12] and Thiele[13] have shown that total cholesterol is not decreased on autolysis of tissues. Schultz's figures, however, nearly always show a loss of a third to a half of the total cholesterol after 4 days' autolysis, and in one case of blood and liver, a loss of two-thirds. This loss is not mentioned in his discussion of his results. It is hard to explain so much loss, even by incomplete extraction, but no other explanation is apparent. The figures of Cytronberg do not show this decrease, but his analyses of fresh blood are most peculiar in that they show only about 30 per cent of the total cholesterol to be in the free condition. From analyses of whole blood, together with analyses of plasma, he calculates in two instances that the red cells contain about 60 per cent of their total cholesterol as esters. Most analyses available for reference place the free cholesterol in whole blood at between 60 and 70 per cent of the total. The figures given below for fresh blood illustrate this well. Furthermore, it is now generally recognized that the red cells contain cholesterol esters only in traces, if at all. Apparently, then, the analytical results in both papers are open to criticism.

EXPERIMENTAL.

All blood and tissue used were obtained as fresh as possible, and always used within an hour. Blood was diluted with an equal volume of water before autolysis. In mixtures of liver and blood, the finely ground liver was mixed with an equal weight of blood, and one volume of water was added. For preservatives sodium fluoride, chloroform, tricresol, and toluene were used, in various experiments. It may be said that in some of the experiments, in which liver was autolyzed, there was apparently so

[11] Gardner, J. A., and Lander, P. E., *Biochem. J.*, 1913, vii, 576.
[12] Corper, H. J., *J. Biol. Chem.*, 1912, xi, 44.
[13] Thiele, F. H., *Biochem. J.*, 1913, vii, 275.

much putrefaction, in spite of a reasonable amount of preservative, that positive results would have been questioned, even if obtained. The effort was made in all the work to use the minimum amount of antiseptic effective to prevent putrefaction, in order to avoid enzyme inhibition as far as possible. In most cases extraction has been carried out with boiling alcohol, but in a few cases ether has been used after drying with sodium sulfate. Such results are probably too low, but, even so, marked hydrolysis should have been apparent. The alcoholic extracts are concentrated, diluted with water, and extracted by ether. The ether extract is divided into equal parts, one-half saponified with alcoholic potash for 2 hours, and again extracted. After evaporation of the ether from both fractions, each is taken up in 10 cc. of alcohol and precipitated by digitonin. The weight of the digitonin-cholesteride multiplied by 0.2431 gives in one case the free cholesterol and in the other the total.

The figures in the column headed "ratio" are obtained by dividing the percentage of total cholesterol in the material by the percentage of free.

DISCUSSION.

Slight variations in the results obtained for total cholesterol before and after autolysis are attributable to experimental error in the method. In none of the experiments is there any such evidence of hydrolysis as has been reported by Schultz and Cytronberg. In one experiment quoted by the latter the ratio of free to total cholesterol rose from 36.6 to 91.7 during 4 days' autolysis, and other figures were almost as striking. It can only be concluded from the figures here quoted, that a careful repetition of their work has entirely failed to confirm their results.

It is hoped that, later on, further work by different methods may throw some light on the main question of whether or not the tissues during life can hydrolyze the esters of cholesterol.

Material	Amount	Total.	Free.	Ratio.	Remarks.
	cc.	per cent	per cent	per cent	
Ox blood, fresh.....	20	0.120	0.081	67.5	$CHCl_3$ and NaF.
4 days' autolysis	20	0.108	0.084	77.7	
Ox blood, fresh.....	20	0.099	0.050	50.51	$CHCl_3$ and NaF.
4 days' autolysis.....	20	0.094	0.047	50.00	
Sheep blood, fresh....	20	0.051	0.031	61.54	
4 days' autolysis.....	20	0.056	0.032	56.90	$CHCl_3$ and NaF.
4 " "	20	0.057	0.033	57.63	NaF.
Dog blood, fresh	20	Lost.	Lost.		
4 days' autolysis	20	0.123	0.081	65.66	$CHCl_3$ and NaF.
4 " "	20	0.131	0.082	62.76	NaF.
Fresh.....	20	0 169	0.102	60.11	Same dog bled 3 days later.
Horse blood, fresh....	20	0.100	0.065	65.00	
4 days' autolysis.....	20	0.099	0.060	60.54	$CHCl_3$ and NaF.
	cc.				
Sheep blood and liver, fresh .	25	0.242	0.125	51.82	Measurement of amount taken only approximate.
4 days' autolysis.....	25	0.300	0.162	53.93	NaF, tricresol, and $CHCl_3$.
Horse blood and liver, equal parts, fresh..	25	0.123	0.107	87.01	
4 days' autolysis.....	25	0.116	0.105	90.65	NaF and $CHCl_3$.
	cc.				
Horse blood citrated, fresh.....	20	0.108	0.087	80.55	Ether extraction. Note increase in total amount extracted after autolysis.
1 month's autolysis...	20	0.132	0.082	62.15	Tricresol.
Horse blood, defibrinated, fresh.....	20	0.114	0.092	80.70	Tricresol.
1 month's autolysis...	20	0.159	0.102	64.77	"
		gm.	gm.		
Horse blood and liver, equal parts, fresh..		0.1086	0.1033	95.1	Ether extraction.
4 day's autolysis.....		0.0399	0.0036	90.9	Tricresol and toluene.

LIPOIDS AS INHIBITORS OF ANAPHYLACTIC SHOCK.

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LIPOIDS AS INHIBITORS OF ANAPHYLACTIC SHOCK.

Studies on Ferment Action. XVIII.*

By JAMES W. JOBLING, M.D., and WILLIAM PETERSEN, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The refractory period following anaphylactic shock, noted early in the study of anaphylaxis, has been variously explained. Among immunologists the idea seems to be generally accepted that the condition is due to an exhaustion of the specific immune substance which together with complement is supposed to act upon the introduced antigen.

Rusznjak (1) first noted that immediately following anaphylactic shock, especially if protracted or following an extended latent period, a definite rise in the antitryptic titer of the animal's serum occurred. Rusznjak, assuming that the hypothesis of Rosenthal (2) in regard to the nature of serum antitrypsin was correct, interpreted his experiment as a demonstration of the splitting of proteins during anaphylactic shock. He furthermore advanced the idea that the period of resistance following shock was due to this increase in the antiferment property of the blood. Seligmann (3) could not confirm these findings, but Pfeiffer and Jarisch (4) later found the observations to be correct, and furthermore showed that a similar rise in antitrypsin occurred following various protein intoxications,—hemolysins, protein split products, etc. Zinsser (5) has recently shown that an increased resistance is found to anaphylatoxins following a first sublethal injection, and we have noted a similar condition with serotoxin (6). Following a sublethal serotoxin injection a well marked rise in the antitryptic titer is observed.

The observation of Rusznjak is therefore true not only for anaphylaxis but probably for every intoxication accompanied by cellular destruction. His conclusions, however, being based on the erroneous theory of Rosenthal as to the nature of serum antitrypsin, are incorrect. We have recently demonstrated that the unsaturated lipoids of the serum are the substances upon which the antitryptic property depends (7). The increase in antitrypsin observed in these cases cannot be due directly to the protein split products, but is to be explained by

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the liberation of lipoids following cellular destruction. The observations of Rusznjak and of Pfeiffer and Jarisch offer then no direct evidence that protein splitting occurs during anaphylactic shock, but merely indicate that a general cellular intoxication has occurred.

The increase in antiferment might nevertheless explain the increased resistance to a second injection of antigen in a sensitized animal. This question can find a solution only if it is possible to increase the antiferment in the blood serum by some means other than a protein intoxication, for following such injury the objection can be made that there is a reduction or exhaustion of both specific and non-specific proteases or antibodies responsible for the shock, together with a destruction of complement. That complement, however, can have no relation to the phenomena, if we regard the intoxication as purely protein, is evident from the fact that the complementary action is not proteolytic but probably lipolytic, a subject which we have briefly discussed in a previous paper (8). Serum antitrypsin being lipoidal and capable of isolation from the serum by means of lipoidal extractives should on reinjection into experimental animals cause an increase in the antiferment property of the blood serum. Such an experiment can be made as follows.

METHODS OF INCREASING THE ANTIFERMENT IN THE BLOOD.

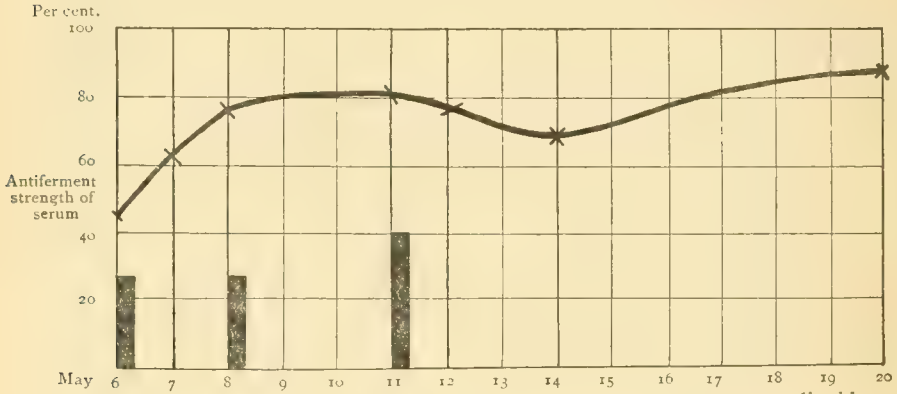
About 300 milligrams of serum lipoids extracted at various times from human and guinea pig serum were dissolved in six cubic centimeters of olive oil. Of this solution a large guinea pig received subcutaneous injections as follows:

May 6, 1914. 1.5 c.c.
May 8, 1914. 1.5 c.c.
May 11, 1914. 2.0 c.c.

The antiferment index, determined by the method discussed in our former papers, is shown in text-figure 1, in which the inhibition of tryptic digestion by 0.05 of a cubic centimeter of serum both before and after the injections is outlined. It will be noted that there is a distinct and prolonged rise from the original 45 per cent. inhibition to more than 80 per cent. as determined on May 11.

Olive oil injected subcutaneously in equal amounts had no effect on the antitryptic index.

Inasmuch as it is difficult to secure large amounts of serum lipoids we have substituted the fats prepared from egg yolk which contain large quantities of unsaturated lipoids. These were obtained as follows: The yolk was extracted twice with alcohol and twice with ether. The extracts were combined and evaporated to dryness. The mass was then extracted with ether and precipitated with acetone. Only the acetone-soluble lipoids were used. As so obtained the lipoids are semifluid and can be injected without the



TEXT-FIG. 1. Increase in antitrypsin following injection of serum lipoids subcutaneously.

use of a solvent. The increase in antitrypsin following the subcutaneous injection of one cubic centimeter in a guinea pig is shown as follows:

	Inhibition by serum before injection. June 29, 1914.	After injection. July 3, 1914.
0.10 c.c. serum	69 per cent. inhibition	79 per cent.
0.075 c.c. serum	44 per cent. inhibition	70 per cent.
0.05 c.c. serum	10 per cent. inhibition	70 per cent.
0.025 c.c. serum	0 per cent. inhibition	0 per cent.

The guinea pig was of medium weight. It was bled from the heart (two cubic centimeters) on June 29, and the egg fat injected the same day. The rise in antiferment is marked.

THE INHIBITION OF ANAPHYLACTIC SHOCK.

Having established the fact that these lipoids will, when subcutaneously absorbed, cause an increase in antiferment, we investigated the effect of such injections on the reaction of sensitized guinea pigs when the specific antigen was injected intravenously.

Six guinea pigs were sensitized on May 16, 1914, with 0.1 c.c. of horse serum intraperitoneally. Three of the animals received subcutaneous injections of serum antitrypsin dissolved in olive oil. The injections, 1 c.c. each, were made on May 21, May 26, and June 5. Purified horse serum albumen solution (1 per cent.) was used for reinjection. The toxic dose of this was determined on the three control animals and resulted as follows:

Animal No.	Weight	Dose.	Dose per gm. weight	Result.
1	240 gm.	0.8 c.c.	0.0033 c.c.	Convulsions, respiratory spasms. Final recovery in 10 min.
2	280 gm.	1.4 c.c.	0.005 c.c.	Death in 2 min. Typical.
3	300 gm.	1.5 c.c.	0.005 c.c.	Same.

The lethal dose, 0.005 c.c. per gram weight, was now injected into the antitrypsin guinea pigs.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Remarks.
4	210 gm.	1.0 c.c.	0.0047 c.c.	Scratches. No convulsions, no respiratory spasms. Remained well.
5	300 gm.	1.5 c.c.	0.005 c.c.	Death in 3 min. Typical.
6	220 gm.	1.1 c.c.	0.005 c.c.	No symptoms except restlessness. Remained well.

In two of these guinea pigs the acute shock had been completely averted. A similar and even more striking effect was obtained with guinea pigs treated with egg fats.

Ten guinea pigs were sensitized with horse serum on July 1, 1914. Six of the animals were injected previously with 1 c.c. of egg fat, on June 29. One of the animals was killed on July 3 and the antitryptic index determined, the result being shown on page 470. They received further injections of 1 c.c. each on July 10 and 16. The effect of the reinjection of a 1 per cent. horse serum albumen solution is shown in the following table.

CONTROL ANIMALS.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
1	170 gm.	0.425 c.c.	0.0025 c.c.	No effect.
2	230 gm.	1.15 c.c.	0.005 c.c.	Marked respiratory convulsions and spasms. Recovered in 30 min.
3	295 gm.	2.95 c.c.	0.01 c.c.	Death in 2 min. Typical.
4	275 gm.	2.75 c.c.	0.01 c.c.	Death in 2 min. Typical.

The minimum lethal dose was therefore 0.01 of a cubic centimeter per gram weight of guinea pig. The injection into the animals treated with egg fat resulted as follows:

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
5	360 gm.	3.6 c.c.	0.01 c.c.	Immediate respiratory spasms; gradual recovery. Well after 10 min.
6	290 gm.	2.9 c.c.	0.01 c.c.	Scratched; no other symptoms.
7	235 gm.	2.35 c.c.	0.01 c.c.	No symptoms.
8	190 gm.	2.37 c.c.	0.0125 c.c.	No symptoms.
9	240 gm.	4.8 c.c.	0.02 c.c.	Marked respiratory spasms, with dyspnea for 20 min. Complete recovery.

It will be observed that all the animals receiving from one to two times the lethal dose made a complete recovery, and in three of the guinea pigs no symptoms of note were observed. The animals remained well during the next two weeks while under observation. There can then be no question but that the increase in antiferment is able to protect the animal from at least twice the minimum lethal dose of antigen and that this increase in antiferment following a protein shock must have a large share in the resistance to a second injection.

THE INFLUENCE OF LIPOIDS PRESENT IN THE ANTIGEN ON ANAPHYLACTIC SHOCK.

It has been found difficult to induce acute anaphylactic shock by means of whole bacteria, and differences have been noted in the period of time following a second injection of various antigens before symptoms of shock would be elicited, the latent period varying from one to fifteen minutes. A long latent period is common with egg albumen; indeed, Rusznjak employed egg albumen for this reason in his experiments. Inasmuch as we have recently demonstrated (9) that bacteria contain unsaturated lipoids which represent the antiferment and that they resist digestion in a degree proportional to the amount of the lipoids present; and since egg albumen resists digestion by means of the ordinary tryptic ferment unless first acted upon by pepsin in an acid medium, whereby the antiferment is destroyed because of alteration in the colloidal dis-

persion brought about by the change in reaction, we next examined the relative toxicity of an antigen before and after chloroform extraction. For this purpose we used the same preparation of purified horse serum albumen, the method of preparation of which we have discussed previously (6).

Fifty cubic centimeters of a 1 per cent. solution were prepared, of which twenty-five cubic centimeters were thoroughly shaken with chloroform and incubated for forty-eight hours, the flask being shaken at intervals. No autolysis occurred during this time. Before use the chloroform was freed from the serum albumen solution by centrifugation and filtration through a coarse paper filter. Guinea pigs were sensitized with horse serum intraperitoneally on April 13, 1914. The minimum lethal dose of the original horse serum albumen solution was determined as follows:

May 20, 1914.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
1	300 gm.	3.0 c.c.	0.01 c.c.	Typical anaphylactic shock. Death in 2 min.
2	300 gm.	1.5 c.c.	0.005 c.c.	Same.
3	360 gm.	0.9 c.c.	0.0025 c.c.	Recovered.
4	310 gm.	0.77 c.c.	0.0025 c.c.	Typical death in 3 min.
5	270 gm.	0.32 c.c.	0.0012 c.c.	Scratched; no other symptoms.

The minimum lethal dose was therefore about 0.0025 of a cubic centimeter per gram weight. The extracted serum albumen solution was toxic in a much smaller dose, as will be seen from the following table.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
6	290 gm.	0.72 c.c.	0.0025 c.c.	Death immediate and typical.
7	290 gm.	0.36 c.c.	0.0012 c.c.	Death immediate and typical.
8	320 gm.	0.2 c.c.	0.0006 c.c.	Death immediate and typical.
9	300 gm.	0.1 c.c.	0.0003 c.c.	Marked convulsions; respiratory spasms. Final recovery.

This experiment would indicate that the lipoids combined with the protein antigen may exert a considerable influence on the relative toxicity of the anaphylactic antigen, and probably explains

the difficulty encountered in obtaining uniform results with such antigens as bacteria which are relatively well protected by their lipoidal components.

THE PREVENTION OF ANAPHYLACTIC SHOCK BY THE SIMULTANEOUS INJECTION OF SOAP SOLUTIONS.

If by extraction of a lipoid containing protein antigen it becomes more toxic, it would seem reasonable that by adding the extracted lipoids to the same the toxicity should be decreased, or, if added in sufficient amount, completely neutralized. We have shown that this can actually be done with serotoxin (6), and we discussed the reasons for the use of soaps in place of lipoid suspensions. A difficulty is encountered, though, in the use of soaps, in that they will in themselves on injection cause a shock similar to that observed in anaphylaxis, so that great care must be used in working with doses that are sublethal (10). While such soaps, oleates for example, are highly antitryptic, we have noted (7) that when incubated with serum, instead of increasing the antiferment index as might be expected, they actually cause a lowering of the antitryptic titer, possibly because of solution of the serum antiferment in the soap solution. Nevertheless, when added to the anaphylactic antigen, soap solutions are able to render the reinjection harmless within certain limits, as will be observed in the following protocols. We may state, however, that we have not been able to secure these results when whole serum was used as an antigen; the results have been obtained only when a solution of horse serum albumen was used. Whether this depends on the greater stability of the albumens as contrasted with the globulins, as might be indicated in the recent work of de Waele (11), or whether it is due to the fact that the soaps lower the antiferment index when added to the whole serum, in which case the injected antigen would have less protection than before, we cannot state.

Sensitized animals and antigen solutions, the toxic doses of which have been described on page 473, were used. 1.5 cubic centimeters of the original serum albumen solution were mixed with one cubic centimeter of a 1 per cent. solution of sodium oleate and incubated

for ten minutes. The surely toxic dose of the antigen was 0.005 of a cubic centimeter per gram weight.

Weight of animal.	Dose.	Dose per gm. weight.	Result.
300 gm.	1.5 c.c. 1.0 c.c. soap solution	0.005 c.c.	No symptoms.

Similar mixtures were made with the lipoid-extracted serum albumen solution, the lethal dose of which was 0.0006 of a cubic centimeter per gram weight.

Weight of animal.	Dose.	Dose per gm. weight.	Result.
280 gm.	0.2 c.c. 1.0 c.c. soap solution	0.0007 c.c.	No effect.
260 gm.	0.4 c.c. 1.0 c.c. soap solution	0.0015 c.c.	No effect.
260 gm.	1.0 c.c. 1.0 c.c. soap solution	0.0038 c.c.	No effect.

These experiments are simply isolated examples from numerous trials which have always shown the same result. In the last protocol the protection is observed to be ample against five times the minimum lethal dose.

Whether or not this protection by the soaps is a mechanical one, due to the formation of a thin soap membrane about the aggregates of the antigen, whether it is due to the actual antiferment property of the soap, or whether its action depends on changes induced in the cellular membranes of the animal so injected, rendering the cells less permeable to the toxic substances responsible for the symptom-complex, can, of course, not be decided from these experiments. We are, however, inclined to assume that the latter is the explanation, especially in view of the work of Schultz (12), Dale (13), Weil (14), and Coca (15), showing that the origin of the shock is probably cellular and not humoral.

CONCLUSIONS.

1. The antitryptic titer of the serum can be increased by subcutaneous injections of serum lipoids (antitrypsin) and of the lipoids from egg yolk.

2. Animals so injected show a relative immunity to acute anaphylactic shock (two minimum lethal doses).

3. Extraction of lipoids contained in antigens increases the toxicity of the antigen when injected into a sensitized animal.

4. Sublethal doses of soap solutions injected simultaneously with the antigen (purified horse serum albumen) prevent anaphylactic shock.

5. The refractory state following anaphylactic shock is related in part to an increase in the antitryptic titer of the serum.

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